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The Fate and Effects of Contaminants in Estuarine Environments

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Abstract

Estuaries are sensitive environments regarding their response to anthropogenic events. The accumulation of toxic trace elements, such as Cd and Pb, has detrimental consequences on benthic community composition and function that can lead to human health issues when seafood is consumed. Eutrophic water can generate blooms of macroalgae and can induce adverse changes in the structure of an entire food web.

Environmental research is of fundamental importance to understand the sources, fates and effects of contaminants. It will permit the development of remediation strategies to improve sustainable practices. The approach taken in the research presented is focused on the measurement of trace elements in sediment, water and biota, and the application of stable isotopes in the detection of sources of contaminants. A novel set of data on New Zealand estuarine species, not usually screened for pollutants, has been assembled in the context of a number of estuarine systems that have been influenced by a range of anthropogenically generated contamination events. The combined use of diffuse gradient ‘in thin films’ (DGT) and the analysis of $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ in water, are new for New Zealand estuarine systems; the correlations generated will enhance baseline datasets for future environmental studies. This research additionally led to the development of a novel environmental indicator, “Ecohardness”, reflecting the estuarine chemical budget by the analysis of the material strength of cockle shell.

From this research, lead, cadmium and zinc were found to be the most concerning polluting elements in the study location, with levels above sedimentary ANZECC safety guidelines. Phosphorus was found in high

concentration in urban and rural estuaries heavily impacted by agricultural practices. Isotopic analysis confirmed sources of pollution with higher values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ near treated wastewater ponds. The accumulation of contaminants was also associated with river and stormwater inputs and the intensive use of fertilisers. The accumulation of trace elements was specifically associated with metal pathways rather than sedimentary composition. However, metal speciation is of fundamental importance in ecotoxicology and is regarded as more significant than bulk concentrations in water and sediment in the understanding of biological response of organisms to trace elements. Accumulation of trace elements in biota was found to be strongly associated to those that were captured in DGT devices deployed in the water ('DGT-labile' elements). DGT-labile elements can be defined as the "bioavailable" fraction, as this technique uses a hydrogel layer to control the diffuse transport of trace elements in solution, to a binding resin. All labile elements were found in higher concentration in the stream/estuarine environment than the ocean, demonstrating the characteristic of estuaries as a sink for trace elements. Further insight into the speciation of the various elements was permitted by calculating the rate of accumulation in different hydrogel thicknesses. Each element could be classified according to their behaviour ranking from putatively free simple inorganic cations with no resupply from complex ligands (e.g. Cd at Bureta), to fully sustained conditions with a constant degree of resupply of free ions and labile complexes (e.g. Ni, Fe and Cu at Bureta).

The development of a novel environmental parameter "EcoHardness", has application to examining pollutant response in estuarine bivalves. While microhardness has been measured previously in bivalves (cockle shell) and correlated to microstructure, this is the first time that microhardness for the same species was analysed in different estuaries that have different anthropogenic

pollution attributes. The method demonstrated an increase of microhardness in response to a decrease of Ca content, that appeared to be inversely correlated to an increase of trace elements that were associated with the general chemical state of estuaries. Hence, harder shells were associated with more polluted estuarine areas. This parameter could be a valuable tool in the assessment of environmental changes linked to the increase of trace metal pollutants as observed around the world in the last few decades.

This work has extended research into the environmental health of estuaries by combining a number of known analytical methodologies in a novel manner and through the development of a new technology. In addition, the work has examined a relevant trophic cascade of species further providing an insight into ecosystem functioning against a backdrop of human presence.

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Preface

Nō ngā tāmariki tēnei ao, whāngaia, kia puāwai ai

“Un bon ecologist, c’est un type qui voit loin et qui a peu de foi dans le progrès, la science et la technique”

“The sea, the great unifier, is man’s only hope. Now, as never before, the old phrase has a literal meaning: we are all in the same boat.”

Jacques Cousteau

Trying to understand is like straining through muddy water.

Have the patience to wait!

Be still and allow the mud to settle.

Lao Tzu

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1 Chapter One

Introduction

1.1 Estuarine health, contaminant sources and sinks

Trace elements and associated contaminant inputs to New Zealand estuaries were examined to extend our understanding of the “present” environmental state of estuarine systems influenced by differing levels of anthropogenic presence with particular focus on the biogenic pathway of pollutant uptake through trophic cascades. When present in abnormal levels, trace elements are of fundamental interest considering their toxicity for aquatic species as well as for humans. A wide range of techniques such as the measurement of trace elements in labile form (diffuse gradient in thin film) and isotope ratio analyses have been combined to allow us to elaborate an interpretation of the sources and fates of contaminants. In addition, a new technique is developed as a tool to provide wider insight into the ecology of estuarine pollution.

1.1.1 Significance of contaminants related to health effects

Trace metals, also known as heavy metals, are elements naturally present on earth usually in small concentrations in the order of parts per million (Luoma *et al.*, 2008). Essential trace metals (e.g. copper and zinc) are fundamental elements required for the wellbeing of organisms and are implicit in metabolic functioning. For example, copper (Cu) is an essential component of numerous oxidation-reduction enzyme systems (Gutteridge, 1983). Zinc (Zn) plays a role as a cofactor in many enzyme systems (immune response) and plays a vital role in lipid and protein metabolism (Li *et al.*, 2010). Muralisankar *et al.* (2015) showed that Zn, when present at an optimal level, can produce better performance in

mineralisation utilisation influencing growth and survival of freshwater prawn. Non-essential elements, for example cadmium (Cd), lead (Pb) and mercury (Hg) do not play an important role in animal's metabolism. However, Cd may compromise the growth and development of aquatic species (Cañizares-Villanueva *et al.*, 2000; Livingstone, 2001). Virtually, all metals can exhibit toxicity to above certain threshold concentrations whether essential or non-essential. Paracelsus (1496-1541), the 'father' of toxicology said: "All things are poisons, and nothing is not a poison, it is the dose or exposure that makes something safe". For example, when Zn is present in excess in fishes and crustaceans, it can dramatically influence the performance of internal metabolisms such as the reproductive behaviour, the muscle composition and the general growth of the species (Jeng & Sun, 1981; Shiau & Jiang, 2006; Muralisankar, *et al.*, 2015). Heavy metals are not biodegradable and tend to be readily accumulated in living organisms, causing various diseases (Clarkson, 1993). Contaminated food by trace metals and other pollutants can have adverse impacts on human health when consumed (Whyte *et al.*, 2009). In Argentina, elevated concentrations of trace metals were measured in the water column and accumulated in fish tissues (As, Hg and Pb) which can be a threat to human health (Avigliano *et al.*, 2015).

People aware of the risk are concerned about the increased accumulation of these elements in biota because of the imminent probability of humans to be exposed to these elements through trophic transfer. The most important elements in terms of trophic transfer via the food chain are As, Cd, Hg, and Pb. If Cd is consumed in excess by people, it can cause irreversible renal tubular injury, non-hypertrophic emphysema and anaemia (Wagner, 1993; Waisberg *et al.*, 2003). The international agency for Research on Cancer (IARC) has classified Cd as a

human carcinogen (group I) on the basis of sufficient evidence in humans and experimental animals. Cd has been associated with cancer of the lung, prostate, pancreas and kidney.

Methyl mercury is the most toxic and the most important in terms of human safety because of its neurotoxic properties (WHO, 1991). Fish are the primary source of mercury in the human diet (Clarkson, 1993). Nearly all the mercury present in the fish muscle is in the form of methyl mercury (Francesconi & Lenanton, 1992). In humans, the main target of mercury is the central nervous system. Several possible molecular targets of methyl mercury exposure in the nervous system include the blood and brain barrier, cytoskeleton and DNA and RNA synthesis (Castoldi *et al.*, 2001). Prolonged Pb exposure can damage the central nervous system, kidneys, and cardiovascular, reproductive and haematological systems (WHO, 1995). Age is a critical variable in the absorption levels, with adults absorbing 7 to 15% from dietary sources; in infants and children, absorption levels can reach 40 to 50% (Ren *et al.*, 2006). Chronic exposure to Pb can lead to a prolonged disease progression involving distal motor neuropathy, possible seizures and coma. Toxicity varies with the chemical state of many metals, for example the trivalent form of arsenic is more toxic. Epidemiological data has shown that chronic exposure of humans to inorganic arsenical compounds is associated with liver injury, peripheral neuropathy, and increased incidence of cancer of lung, skin, bladder and liver. Several mechanisms have been implicated in arsenic induced genotoxicity, which included oxidative stress. DNA repair inhibition and direct mutagenesis have been reported (Bach *et al.*, 2015; Dutta *et al.*, 2015) and can increase the likelihood of tumour formation.

To protect against the toxic effects of trace metals, a wide range of safety limits of toxic elements have been developed such as acceptable daily intake thresholds, maximum residue level, and maximum limit. It is pertinent to recognise that these levels in human food will frequently reflect environmental condition (Underwood, 2012). In New Zealand, the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC) provide guidance for setting environmentally unacceptable trigger values in sediments, water and food including assessment of human toxicity by way of consumption (ANZECC, 2000).

1.1.2 Levels and sources of contaminants

Since the beginning of the century, the degradation of the environment during the rapid expansion of the industrial age was seen as a low priority, being considered as a low risk: “By sensible definition, any by-product of a chemical operation for which there is no profitable use is a waste. The most convenient, least expensive way of disposing of said waste – up the chimney or down the river- is the best” (Haynes, 1949). As an example, recent research shows that the level of mercury in the global ocean increased 3 fold since industrial revolution (Lamborg *et al.*, 2014). In most cases, the negative influences of human development occur simultaneously and direct correlations with the separate source causes are hard to make.

Cd naturally occurs in Zn and Pb ores; however, the industrial use of the metal and agricultural activities have led to widespread dispersion of this element in the environment and food items (Wagner, 1993; Pérez & Anderson, 2009; Schipper *et al.*, 2011). Studies have shown that the average concentrations of trace elements in the agricultural environment have increased over the years, with cadmium constituting one of the primary concerns. Inorganic mercury or phenyl mercury deposits in aquatic environments from industrial wastes and can be

converted through biological methylation to methyl mercury by microorganisms (Jensen & Jernelov, 1969). The agricultural use of methyl mercury fungicides has also taken its toll on human health. In rural Iraq, more than 6000 cases of severe poisoning and more than 600 deaths were recorded in hospitals throughout the country (Bakir *et al.*, 1973). Industrial and vehicular pollution are primary Pb sources of contamination of air, water, soil, and food. Pb exposure can be further enhanced by domestic food processing, such as food canning, serving of food in glazed pottery and delivery of water by lead pipe. The major sources of exposure to As are food and water. The principal cause of elevated As is the widespread use of As compounds as insecticides, herbicides and defoliants for agriculture. Arsenic also enters the environment by mining and smelting of non-ferrous metals, application of phosphate fertilisers, fossil fuel combustion, and application of municipal sewage sludge.

Focused case studies in laboratories permit segregation of specific factors, yet analyses designed to separate multiple simultaneous environmental impacts in real life situations in the natural world are hard to perform. Unfortunately, the sources, metal speciation phenomena, and sinks of most trace elements are poorly understood due to the high level of complexity of multiple discharge points, the biogeochemical systems that constitute the receiving environment and also due to the insufficient number of field directed studies.

New Zealand was initially colonised primarily for agricultural land rather than for its mineral wealth. After 1840 however, many English immigrants came to New Zealand and started to search for gold and coal. Past long term effects of land use such as mining, forestry and farming were generally ignored and yet these activities can constitute an on-going problem in the future (Druzbecka & Craw, 2013; Nielsen *et al.*, 2015). There were no regulations to prevent or

mitigate negative environmental impacts. Mining operations were carried out with little consideration for future land use, and waste rock and tailings produced during mineral extraction processes were generally deposited in the nearest river or estuary (Druzbecka & Craw, 2013; Niyogi *et al.*, 2013). Clearing forests in steep unstable hill country for farming, such as across the east coast of the north island, has produced large areas where rapid erosion has cultural and economic impact (Marden *et al.*, 2014). Around 1920, agricultural practices came into a new age with the application of new soil science, particularly advancing the use of fertilisers (Dawson & Hilton, 2011). The amount of nitrogen fertiliser used in New Zealand has increased by about 10 fold since 1985. Between 1960 and 1985, the amount of pesticide aerially sprayed on farms increased by 4 fold (MacLeod & Moller, 2006).

New Zealand has been subject to high levels of potentially toxic chemicals in soils in rural areas (and in some of the food produced) which leaches into river and estuarine systems due to a combination of natural and anthropocentric factors. Many contaminants that have human health implications (including cancer) have been identified in oil products, pesticides, fertilisers, industrial chemicals and their by-products, and leachates from waste disposed at landfills. Contaminants include heavy metals (e.g. Cd, As, Zn, Pb, Cu, Cr and Hg), organochlorines (e.g. DDT, DDE, PCP, PCBs and dioxins), solvents, hydrocarbons, asbestos and cyanides. A survey of heavy metal contamination in pastoral soils was conducted in the early 1990s (Roberts *et al.*, 1996). As, Cd, Cu, Pb and Zn were investigated with Cd found at elevated levels, but concentrations were well below the safety ANZECC values. Cadmium levels recorded to date do not appear to represent a threat to soil or animal health; however, cadmium accumulation in the kidneys and livers of sheep and cattle may reach levels exceeding tolerance limits for export markets. In

New Zealand, cadmium concentration in pasture soils is associated with the use of superphosphate fertiliser. Total cadmium showed an increase of around 8 fold in market garden soils relative to natural levels and this increase was a reflection of the high inputs used for crops such as potatoes, lettuces, and onions (Roberts *et al.*, 1995). Another source of heavy metal contamination in rural soils that causes leachate issues is the use of copper and arsenic compounds to control fungi in orchards and vineyards as well as the use of chromated copper arsenate (CCA) as wood preservative (Gaw *et al.*, 2006; Robinson *et al.*, 2006).

Estuaries are now facing increasing pressure from metal contamination because of the elevated discharges from various sources (Hosono *et al.*, 2010; Yang *et al.*, 2015b). Due to their fundamental functions in the survival of species (Day *et al.*, 1989) and their high use in recreational activities, researchers around the world have been assessing the state of estuaries to prevent pollution events and negative cascades of contaminants in ecosystems. For example, negative cascades can be associated with the alteration of natural hydrologic flows, the proliferation of aquatic nuisance species and toxic contaminants affecting the whole ecosystem. Sources of contamination reflected land activities such as mining, uncontrolled use of agrichemicals and urban development (Gimeno-García *et al.*, 1996; Duan *et al.*, 2015; Othmani *et al.*, 2015). In New Zealand, natural events releasing trace metals can often be associated with the emission of products from volcanic vents and geothermal springs (Hedenquist & Gulson, 1992; Wardell *et al.*, 2008). Research on contaminants in estuarine systems have mainly been focussed on sediment analysis in Manukau and Wellington Harbours and the Avon-Heathcote Estuary as arguably the most contaminated estuarine systems in New Zealand (Glasby *et al.*, 1988; Glasby *et al.*, 1990a; Dickinson *et al.*, 1996; Hayward *et al.*, 2004; Stevens *et al.*, 2004; Marsden *et al.*, 2013; Marsden &

Baharuddin., 2014; Moores & Timperley., 2008; Parshotam *et al.*, 2008). Recent research in the Manukau Harbour did not show a decrease in the trend of Zn concentration in surface sediment (Hayward, *et al.*, 2004; Parshotam, *et al.*, 2008). Williamson *et al.* (1996) measured trace metal concentrations in the water column (particulate and dissolved) to undermine the importance of tidal movement and potential re-suspension from surface sediment. They found that the mobilisation of contaminated surface sediment was the main source of metals in the water column. As (total and inorganic), Hg and Pb have been measured in edible seaweed collected in New Zealand (Smith *et al.*, 2010). They found low concentration of these metals except for total arsenic, which was present as a non-toxic form and did not present any risk for human consumption. Past research has identified sources of trace metals. Sources of Pb have been attributed to stormwater runoff, the increase of Zn has been attributed to galvanised surface such as roof and steel mill work and Cu has been found at close distance to old sewer outfalls (Glasby, *et al.*, 1988). High concentration of As, Hg, Pb and Zn above ANZECC guidelines have been identified near a commercial slipway in Napier, mainly associated with paint chips and historical antifoulant paints (Strong, 2005). When compared to these studies, Tauranga Harbour displayed lower metal content in surface sediment and mainly accumulated in the inner areas of the harbour (Park, 2003; Ellis *et al.*, 2013; Park, 2014). Trace metals measured in surface sediments however, showed little difference between rural estuaries with higher concentration of As, Cr and Cu compared to urban estuaries with higher levels of Zn, for example the Waikareao sub estuary in Tauranga (Park, 2014).

Detailed understanding of the sources of trace metals and nutrients in the Tauranga Harbour has not been successfully achieved; however, the Regional

Council has been commonly cited as associating the fluctuations of contaminants with land use (Park, 2003). Nutrients are common contaminants from agricultural use while urban areas often result in higher concentrations of zinc, lead and copper. One of the main sources of contaminants is stormwater especially those localised in commercial areas (e.g. Mount Maunganui). McIntosh and Deely (2001) found higher Zn concentration in sediment adjacent to a commercial stormwater source by 23 fold, when compared to a control drain in Katikati (rural). Additionally, these authors for the same sites measured an increase of Cd, Cr and Cu by 6 fold and As and Pb by 4 fold. Bay of Plenty soil trace elements (As, Cd, Cr, Cu, Pb, Hg, Ni, U and Zn in land sediments) were analysed by the Bay of Plenty Regional Council in 5 differing land use categories namely dairy, maize, sheep/beef, deer and kiwi fruit (Guinto, 2011). The author found elevated concentrations of Cd in dairy pasture sites with 26% of the sites exceeding the ANZECC guideline value. The source of Cd enrichment was associated with the intensive and continual use of phosphate fertilisers. Additionally, this study found an increase of Cu and Zinc concentration in soil after the year 1999 in Kiwifruit orchard sites, most likely a result of copper spraying widely used to control the *Pseudomonas* disease (PSA) of Kiwi fruit vines (Guinto, 2011).

1.2 Research statement and objectives

While the environment is an essential value for many New Zealanders, most of our land activities are based on extensive agricultural practices that have led to a legacy of environmental degradation. Estuaries are the natural receptacles of land waste through leaching and river borne runoff. The fates and effects of these contaminants combined with additional sources of pollutants from urban precincts in estuarine systems are poorly understood due to a high number of sources and subsequent interactions amongst contaminants against a varied biogeochemical backdrop (Fig. 1.1). In this model, a wide range of nutrient and contaminant sources are presented to illustrate the high level of complexity and successive challenges for environmental managers and scientists in the understanding of effects from multiple sources.

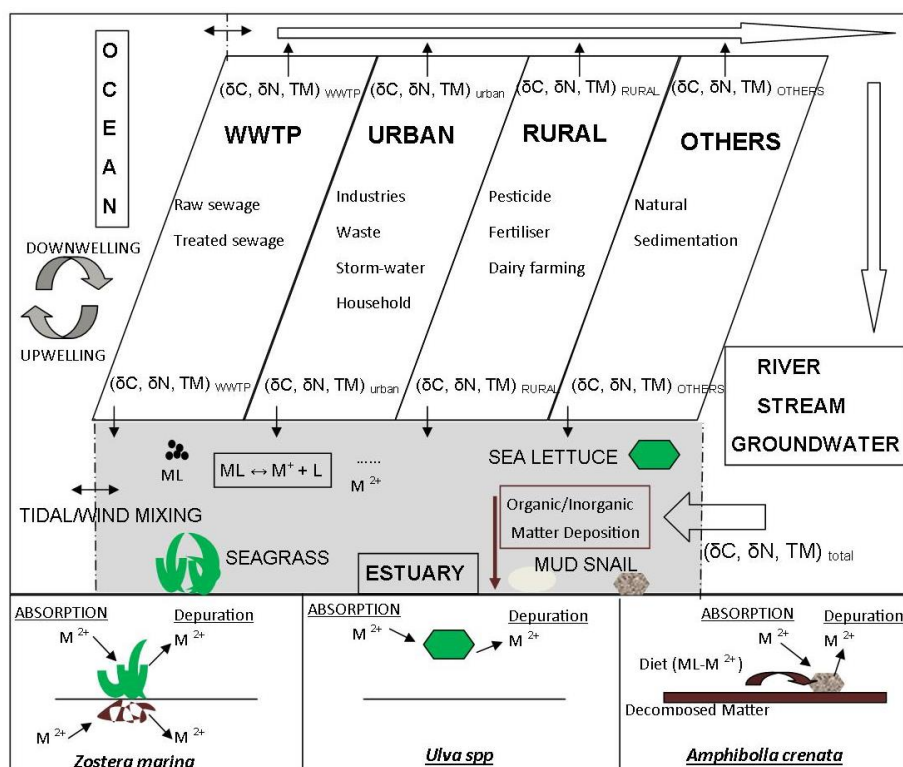


Fig. 1.1. Schematic representation of pollution sources and associated fates in key estuarine species M: metal, L: ligand, WWTP: wastewater treatment plant. (Huteau, unpublished)

Thus the aims of this project were:

- To compare the degree of metal contamination in different environments contrasted by various land uses
- To identify areas with higher trace metals concentrations, and understand their sources and bioavailability across a range of estuarine species from co-occurring but phylogenetically and ecologically distinct taxa
- To compare and contrast monitoring techniques to progress understanding of complex dynamics of the sources and sinks of contaminants in estuarine environments, and to contribute to ‘best practice’ for future studies.
- To identify areas with higher nutrient levels (e.g. nitrogen and phosphorus) and understand their sources and impacts on nutrient requirements, limitations and availability for a range of species across a trophic cascade.
- To examine the use of stable isotopes as a tool to track sources of metal and nutrient contaminants
- To create new techniques to support more accurate and efficient environmental monitoring in the future, including the elaboration of a novel environmental parameter using cockle shells
- To discuss the use of this research to future environmental management and how it can be related to Tangata Whenua in support of their customary rights

Therefore, the primary hypotheses for this research were:

- Estuaries are directly influenced by freshwater input and land activities. Therefore, estuaries will have different and specific elemental characteristics because of a variety of land usage.
- The productivity of primary producers and the survival of benthic species will be affected by these contrasting environments and the proximity of the organisms with contaminant sources.
- Organisms are accumulating trace elements from their local environment. Therefore, stable isotope signature and chemical composition in tissue will be representative of the species affinity to accumulate trace elements, the uptake pathway of contaminants and their local environment.
- Calcium carbonate, a ‘soft element’ (low microhardness) is the principal element constituting bivalve shells. Anthropogenic pressure and the associated release of trace metals will influence the chemical composition of bivalve shell. Thus, variation in shell microhardness value can be used as a marker, tracer or indicator of heavy metal input reflecting local estuarine environmental condition.

1.3 Organisation of the thesis

This thesis is organised into six chapters including the introduction and conclusion sections. The first chapter (introduction) provides a review of toxic metals and their health related effects, as well as general international and national background to metal contamination sources in the environment drawing on present knowledge in the fields of ecology and chemistry. The introductory chapter describes the problem to be addressed and lists the overall objectives.

Chapter 2 assesses trace element contamination in urban and rural estuaries to identify potential pollution sources and their associated ecological and human risks.

Chapter 3 investigates trace elements in water by comparing traditional water quality sampling techniques with passive sampler technologies. The work embodied in this chapter provides information on metal speciation (kinetic) and elaborates on chemical interactions with the biota.

Chapter 4 evaluates nitrogen and phosphorus concentration in Bay of Plenty estuaries as associated with eutrophication levels, nutrient requirements and limitations. This chapter permits elaboration of the use of stable isotope techniques to detect contaminant sources, with emphasis on a wastewater treatment plant (WWTP).

Chapter 5 details a world first development of a new environmental indicator by using the mechanical proprieties of cockle shells as a quantitative indicator of the chemical budget within the estuary.

The final Chapter 6 provides an overall conclusion to the project and brings together a synthesis across the project components. Suggestions for further research are also discussed.

Research chapters (3, 4 and 5) have been prepared in order to permit fast publication in scientific journals after thesis submission. Chapter 2 “Trace element mobilisation from sediments to biota in Bay of Plenty estuaries” has been submitted to Marine Pollution Bulletin.

2 Chapter Two

Trace element mobilisation from sediments to biota in Bay of Plenty estuaries

2.1 Introduction

Estuaries, the physical interface between riverine and coastal environments, are highly productive ecosystems (Costanza *et al.*, 1996). They are important for the ecology of many species including those that move from freshwater to the sea and *vice versa*. Wetlands are particularly important as nursery grounds for native species (Sheaves *et al.*, 2007; Davis *et al.*, 2012), and intertidal flats are a habitat for seagrass and many shellfish such as bivalves and gastropods.

Estuaries are partly enclosed wet areas where water flow is reduced and are considered a natural ‘scrubber’ for the filtration and therefore accumulation of many pollutants (Tack *et al.*, 2007). As such they are also prone to accumulation of anthropogenically derived nutrients, toxic compounds such as polychlorinated biphenyl (PCB), dichlorodiphenyltrichloroethane (DDT), and toxic metals. In particular, increased human activity in coastal regions worldwide has resulted in the marked accumulation of trace metals in sediments in recent decades (Glasby *et al.*, 1990b; Lau & Chu, 1999; Norville, 2005; Kalnejais & Martin, 2006).

Because estuaries display unique biological and physical features with complex chemical interactions between sediment, water and associated organisms it can be difficult to delineate the sources of contamination, or to attribute detrimental effects to the observed accumulation of metals and other pollutants in biota. Sediments are a sink for metals present in the water column which are

removed by deposition of chemical precipitates or by destabilisation of particulate and colloidal phases as riverine waters contact more saline, estuarine waters (Hartland *et al.*, 2013). Hence, sediments may well constitute a secondary source of contamination for the water column by remobilisation and resuspension of particles (Simpson *et al.*, 1998). In addition, through processes of bioaccumulation and biomagnification, the effects of contaminants may be expressed in the higher trophic levels of estuarine food webs. In this regard, species that are consumed by humans such as mud snails (*Amphibola crenata*) (especially for indigenous inhabitants of the coastal estate - in New Zealand known as tangata whenua), may present a significant health threat, yet these species are seldom profiled in bioassays.

Sedimentary trace metal concentrations can be relevant indicators of ecosystem health when monitoring the spatial and temporal pattern of contaminants in the estuarine environment (Buggy & Tobin, 2008; Hosono, *et al.*, 2010). The health and metal content in associated organisms are influenced by the character of sediments (Cheggour *et al.*, 2001; Hewitt *et al.*, 2005; Fukunaga & Anderson, 2011). However, most studies to date, especially in New Zealand, have examined the correlation of estuarine organism demography solely with the physical character of sediments (Lelieveld *et al.*, 2004; Lambert *et al.*, 2007). Alternatively, reports link pollutant profiles in a few targeted species without reference to ambient sediment chemistry or the wider trophic relationships of the bioindicator organism (Burggraaf *et al.*, 1997; ANZECC, 2000; Peake *et al.*, 2006).

Relatively little has been published on the likely chemical interactions of trace metals with sedimentary biogeochemistry in differing parts of an estuarine

system, and in turn, how that influences organism ecology. Bivalves and gastropods are suitable indicator species for use in the monitoring of toxic metals, as they ingest both detrital organic and inorganic particulate material (Zuykov *et al.*, 2013), and bivalves can concentrate metals significantly above ambient levels (Magos, 1994). In a complex estuarine environment, accumulation of metals by higher organisms is affected by the metal content in sediment as well as variables such as metal speciation, pH and redox potential in the surrounding medium. This in turn influences the mechanisms and magnitude of transport processes governing the passage of metals into cells and their eventual retention or excretion (Campbel, 1995). The presence of contaminants such as metals can decrease the efficiency of biological reactions generating potential adverse effects, which in most cases are hard to determine due to the lengthy time spans involved in chemical accumulation and eventual effects on organisms. In seagrass, for example, higher concentrations of Cu and Zn decreased chlorophyll *a* fluorescence (Macinnis-Ng & Ralph, 2002). Accumulation of trace metals (Cd, Cu, Pb and Zn) are toxic to molluscs, with different species exhibiting different degrees of tolerance (Chan, 1988). Furthermore, the size of the organisms also affected their tolerance, and thus survival rates. An experimental study looking at Cd, Cu, Pb and Zn toxicity on larvae of a bivalve species (*Crassostrea gigas*) found that these metals were bioavailable and could inhibit normal growth, with this effect being most marked for Pb (Fichet *et al.*, 1998). In humans, metal accumulation can lead to a wide range of detrimental effects, such as carcinogenicity, mutagenicity and teratogenicity (Thomas *et al.*, 2009).

General ecological approaches taken to assess metal toxicity in order to examine ecosystem health are based on comparing the concentrations of metals measured in sediment and fauna to critical guideline levels, thus allowing risk

assessments to be made as well as estimation of potential toxicity toward both estuarine organisms and human consumers (Long *et al.*, 1995; ANZECC, 2000; FSANZ, 2008). Because diet is not the only factor responsible for metal accumulation, overall bioaccumulation is likely to be underestimated. For example, absorption through the epidermis and ingestion from water are likely to be important mechanisms by which metals enter organism tissues. However, by definition, all metals and metalloids present in the environment have potential toxic consequences to organisms once a certain biotoxic threshold is exceeded. Finally, the toxicity of metals originating from natural processes may be distinct from those arising from industrial practices because of the varied speciation of metals emitted, e.g. organometallic compounds and metal nanoparticles. Thus, anthropogenic metals may be assimilated by organisms via different pathways and may have distinct mechanisms of toxicity than metal species of natural origin, e.g. those complexed by natural inorganic and organic ligands.

This study reports trace metal concentrations in the sediments and fauna of five New Zealand estuaries: Waimapu, Rangataua and Waikareao in the southern “urban” Tauranga Harbour, Tuapiro as representative of a rural estuary in the northern part of the Tauranga Harbour, and Ohiwa Harbour, a typical rural environment characterised by a high proportion of native forest in its catchment (Fig. 2.1). In the last 20 years, Tauranga Harbour and most of its’ sub-estuaries had a loss of biodiversity as indicated by a decline in seagrass habitat (Park, 1999), and a simultaneous increase in abundance of fast growing opportunistic macroalgae species such as *Ulva* spp. (Park, 1996; Barr, 2007). From 1959 to 2011, sea grass abundance declined by 34% in the southern part of the Tauranga Harbour and 14% in the northern part (Park, 1999). In Ohiwa, sea grass abundance has also declined by 10% between 1945 to 2011. There have also been

increases in mangrove (*Avicennia*) forest in both harbour systems (Lundquist *et al.*, 2014).

In Tauranga harbour, the southern sub-estuaries are intertidal sand flats and are shallow with a constricted oceanic entrance limiting the efficiency of tidal flushing, resulting in longer water residence times (Tay *et al.*, 2012) and an elevated rate of sediment deposition (Hancock *et al.*, 2009). These shallow estuaries are therefore likely to be more sensitive to pollution given that particulates are not efficiently removed to the ocean. Ohiwa Harbour is subject to much less anthropogenic contamination and is flushed more efficiently than the shallow sub-estuaries of Tauranga Harbour. For the purposes of this research, representatives of a typical New Zealand estuarine food web were selected as study species reflecting a trophic range of relevance: sea lettuce (*Ulva lactuca*), seagrass (*Zostera marina*), cockles (*Austrovenus stutchburyi*) and mud snails (*Amphibola crenata*).

The concentration of twelve trace elements in these organisms was evaluated in five estuaries that were mainly differentiated by the degree of human presence and land use practices as described above. These elements include metals and semi-metals (V, Cr, Ni, Cu, Zn, As, Fe), toxic or non-essential elements (B, Al, Cd, Pb, U) and a non-metals (P).

The aims of this research were: 1) to compare the degree of metal contamination in selected plant, algae and animal species to New Zealand sediment quality guidelines (SQG) and the food standards of Australia New Zealand (ANZECC, 2000; FSANZ, 2008); 2) to identify the source localities of trace metals and understand their fates in the estuarine ecosystem; 3) to examine

the affinities of species to accumulate different elements; and 4) to create baseline values for future monitoring of metal contamination status of the estuaries.

We hypothesise that trace elements in sediment and biota will be higher in the inner part of estuaries due to the presence of freshwater input from populated and farmed catchments. Additionally, differences in metal accumulation between species will be found due to different pathways in metal uptake. Seagrass will have higher metal content than sea lettuce due to the uptake of contaminants from the sediment through the rhizome.

2.2 Method

2.2.1 Study sites

Five estuaries were surveyed within Tauranga and Ohiwa harbours located on the east coast of the north island of New Zealand, in October 2013 with samples taken at three different sites within each estuary system (Fig. 2.1). Sample locations were selected based on their proximity to likely sources of contamination (e.g. river entry points) and two more distant locations, in order to integrate the range of physical, biological and chemical changes likely to be occurring. In addition, the sites selected within each estuary have traditionally been used for cultural harvesting of seafood. The estuaries have been further grouped into two general categories based on the degree of human influence. The first group (urban) included Waimapu, Waikareao and Rangataua estuaries. The second group (rural) comprised the Tuapiro and Ohiwa estuaries.

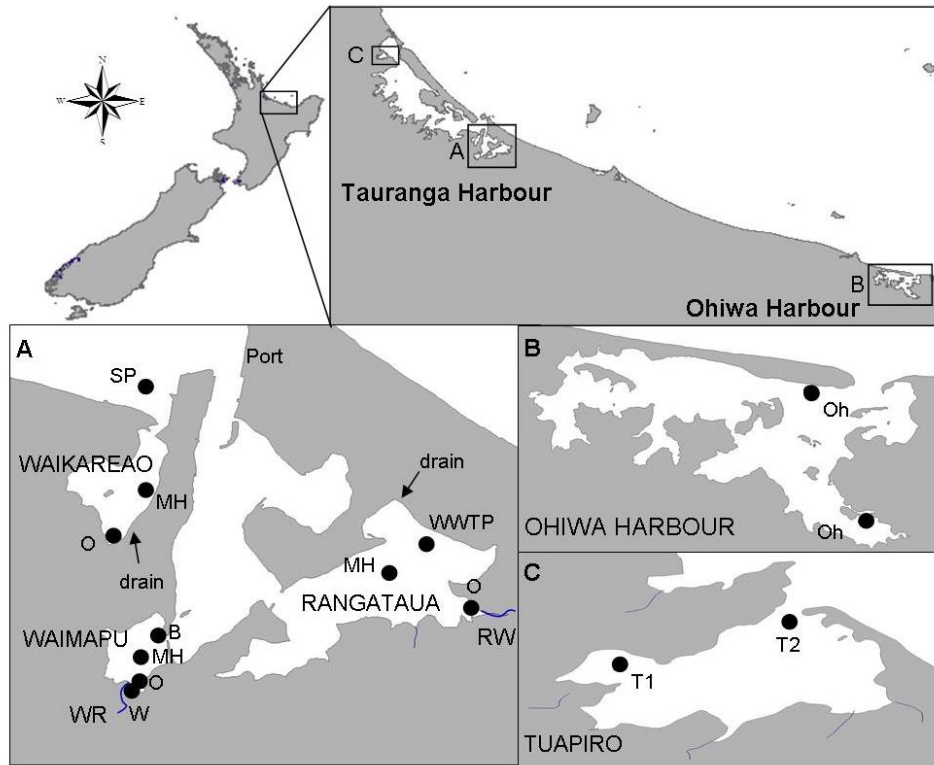


Fig. 2.1. Sampling locations (circles) in Ohiwa and Tauranga Harbours. W: wetland, O: river outlet, MH: middle harbour, B: bridge, SP: Sulphur Point, WWTP: wastewater treatment plant, WR: Waimapu River, RW: Waitao River, drain: stormwater outlet.

Tauranga Harbour

The Tauranga Harbour, one of New Zealand's largest estuaries, covers an area of 210 km² and is protected along its seaward border by a raised sand-barrier island. The southern part of Tauranga harbour is highly fragmented due to a variety of land use practices. It is surrounded by a moderate population of 115 000 people (NZ statistics, 2013), a large commercial port, burgeoning industry, wastewater ponds and with significant agricultural activity across catchments, all possible, impacting the marine environment (Fig. 2.1). The studied estuaries were selected as they are the closest to denser human activities and showed similarities regarding biodiversity and the types of land use in their respective catchments (Table 2.1). The Waimapu River is the second most degraded fresh water waterway in the Bay of Plenty with regard to nutrient levels, mainly affected by intensive agriculture and urban practices (Table 2.1). In the Rangataua Estuary, similar issues of intensive agricultural activity are polluting the Waitao River, the

main source of freshwater input (Table 2.1). Furthermore, wastewater treatment ponds (WWTP) are localised on the edge of the Rangataua Estuary and potentially bring more contaminants into the ecosystem. Waikareao Estuary has similar land use within the catchment as the other urban habitats; however many industries including the production, handling and application of chemicals are situated at the head of the Waikareao Estuary with a Port and Marina at its entrance. Tuapiro Estuary is located in the northern part of the Tauranga Harbour, and was selected for comparison as representative of a rural habitat with semi-intensive agricultural activities in the lower hinterland but restricted city development.

Ohiwa Harbour

The Ohiwa Harbour covers an area of 26.4 km² and is considered as one of the most natural harbours in New Zealand with high conservation values and outstanding wildlife features. Two sites in Ohiwa Harbour were selected as representative of a rural environment with minimal urban impact but with forestry present in the catchment potentially increasing sediment runoff (Suryatmojo, 2015) (Table 2.1). The first site was located on the open seaward side at the end of Ohope sand spit. The second site was located in the inner part of the harbour near Kutarere (Fig. 2.1).

Table 2.1. Land use (%) in estuarine catchments of the Tauranga and Ohiwa harbours, Bay of Plenty, New Zealand (Shaw et al., 2010).

| | Rangataua | Waimapu | Waikareao | Tuapiro | Ohiwa |
|---------------------|-----------|---------|-----------|---------|-------|
| Indigenous forest | 17.7 | 30.7 | 39.2 | 51.7 | 20.5 |
| Wetland | 1.2 | 0.5 | 0.3 | 0.3 | 1.8 |
| Exotic forest | 12.5 | 6.1 | 15.5 | 3.3 | 18.6 |
| Orchards and crops | 5.7 | 5.1 | 4.9 | 15 | 0.5 |
| Pastoral lands | 39.5 | 46.2 | 30.4 | 28.8 | 46.9 |
| Exotic vegetation | 1.5 | 1.2 | 0.4 | 0.4 | 0.1 |
| Surface mine | 0.4 | 0.1 | 0.1 | - | - |
| Urban | 21.2 | 9.9 | 9.2 | 0.4 | 1.1 |
| Catchment size (ha) | 9862 | 13029 | 17330 | 6622 | 17511 |

2.3 Collected species

In general, biodiversity in estuarine ecosystems is high, with species ranging from microscopic organisms to apex predators such as sharks and kingfish. Estuarine species occupied a range of habitats including: the water column with fixed and free macroalgae (e.g. sea lettuce), the seabed with benthic species (e.g. seagrass an angiosperm, and deposit feeders such as mud snails), and sediment infauna (e.g. cockles). The mechanisms of contaminant uptake to each species will differ due to different feeding strategies with multiple possible pathways for contaminants to enter each organism. Modes of contaminant uptake may not necessarily be consistent throughout phylogenetic groupings. For example, mud snails fed on the microphytobenthos and bacteria while cockles are filter feeders feeding on the microscopic plankton that are floating in the water column. This will influence trace metal uptake, accumulation rates and steady-state concentrations.

For this research, we focused on species selected for their accessibility for sampling, mobility, diet and use for human consumption. We chose two potentially competing primary producers, seagrass (*Zostera marina*) and sea lettuce (*Ulva lactuca*), a filter water feeder known as cockle (*Austrovenus stutchburyi*) and a deposit feeder known as mud snails (*Amphibola crenata*). Briggs (1972) classed mud snail adults as larger than 20 mm, and here they are separated into 2 classes with mudsnails smaller than 20 mm (MS< 20 mm) and mudsnails larger than 20 mm (MS> 20 mm), to examine any size related effects. A total of six mud snails and cockles were respectively pooled together to make one sample with three replicates collected per site.

2.3.1 Trace element analysis

At each site, 10 g of surface sediment was collected with a syringe. Six subsamples of surface sediment per site were pooled to create a composite sample with three replicates collected at each location. Samples were freeze dried and sieved at 64 μm in the laboratory. The whole tissues of mud snails and cockles, sea lettuce thalli and seagrass leaves were freeze dried and ground with a mortar and pestle. Fine sediment (<64 μm) and biological species were analysed for trace elements by inductively coupled plasma mass spectrometry (ICP-MS). An acid digestion procedure followed the US EPA Standard Method 2002 for all samples. This involved adding 4 ml of concentrated HNO_3 diluted 1:2 and 10 ml of concentrated HCl diluted 1:5 to 1 g of sample. Samples were left overnight to digest. The following day, samples were heated on a hot plate for 45 minutes at a reflux temperature of 75°C. The entire sample was then diluted to 250 ml, and left to stand overnight. 15 ml of solution was filtered prior to being placed in 15 ml tube for ICP-MS analysis. Metals selected for further analysis were K, B, Al, V, Cr, Fe, Cd, Ni, Cu, Zn, As, U and Pb and the non-metal P. Flush blanks were carried out every 8 samples analysed to monitor any instrument drift. Five certified standards were used at the beginning and undertaken following every 24 samples to investigate the accuracy, precision and repeatability of the ICP-MS method (Merck XXI 50ppb, Merck IV 50ppb, Merck SCP 5000ppb, Merck P 5000ppb, Merck Hg 10ppb). Water reference standards (SLRS-4) were analysed for validation. The analysis of the reference standard revealed satisfactory recovery for all elements and were lower for those present in smaller concentrations in the reference sample (Table 2.2).

Table 2.2. Detection limit (ppb) and metal recovery (%) for trace elements analysed by ICP-MS

| | B | Al | P | Ca | V | Cr | Fe | Mn | Ni | Cu | Zn | As | Cd | Ba | Pb | U |
|-----------------------|----|----|----|----|----|-----|----|-----|-----|-----|----|----|------|-----|-----|------|
| Detection Limit (ppb) | 5 | 3 | 20 | 50 | 2 | 0.5 | 20 | 0.5 | 1 | 0.5 | 1 | 1 | 0.05 | 0.5 | 0.2 | 0.02 |
| Metal Recovery (%) | 94 | 99 | 96 | 90 | 87 | 98 | 88 | 94 | 101 | 95 | 98 | 94 | 91 | 97 | 95 | 87 |

2.3.2 Assessment of metal contamination

Enrichment factor (EF)

The enrichment factor (EF) was calculated (as below) permitting an assessment of the degree of metal pollution affecting each estuary. Putative ‘pre-anthropogenically’ derived (natural) metal concentrations were required to provide a comparatively ‘pristine’ control background. Values are derived from average metal concentrations of the relevant continental shale (Turekian & Wedepohl, 1961). The EF estimates the anthropogenic effects as follows (Buat-Menard & Chesselet, 1979):

$$EF = (Ms / Als) / (Mr / Alr) \quad (1)$$

where Ms and Als is the concentration of metal studied (Ms) and metal of reference (Als) in the sample; Mr and Alr is the concentration of metal studied and reference metal in the continental shale. In this study, averaged values of aluminium for the six estuaries have been chosen as the reference metal due to its greater abundance and lower variability in the local environment than Mn and Fe. Six degrees of contamination are commonly defined: $EF < 1$: no enrichment, $1 < EF < 3$: minor enrichment; $3 < EF < 5$: moderate enrichment; $5 < EF < 10$:

moderately severe enrichment; 10>EF>25: severe enrichment; EF>25: very severe enrichment.

Sediment and seafood guidelines

A sediment quality guideline (SQG) was derived from the Australian and New Zealand Environment Conservation Council (ANZECC) guideline (Table 2.2). In general, ANZECC set similar values to the National Oceanic and Atmospheric Administration (Long *et al.*, 1995), but are less high for Cu, Zn, Cd and Pb at their lower limits. In this report, two sets of values are considered: the effects' range low (ERL) and the effects' range median (ERM).

Seafood guidelines were obtained from the Food Standards Australia New Zealand (FSANZ, 2008) setting the maximum acceptable concentration for As at 1 mg/kg and for Cd and Pb at 2 mg/kg wet weight tissue. These thresholds are less conservative than those set by the European Commission which state a value of 1 mg/kg for Cd and Pb (Commission, 2001). While no environmental threshold concentrations have been set for Cu and Zn in New Zealand, generally expected levels (GELs) have been chosen for Cu and Zn on the basis of observed concentrations in commercial seafood thereby providing a value for remedial action if a level is exceeded (FSANZ, 2008). These values are based on the calculation of median/90th percentile, which are 3-30 mg/kg for Cu and 130-290 mg/kg for Zn. In this paper, we refer to metal abundance in dry weight. The safety guideline concentrations (wet weight) have therefore been calculated by applying species specific weight conversion factors.

Bio-indicator Factor (BF)

Bio-indicator factors for species have been calculated to estimate the degree of accumulation of a metal from their diet compared to surface sediment

(Equation 2). These values are also used to examine the degree of correlation between sediment and tissues in different sampling locations (Vassiliki & Konstantina, 1984) :

$$BF = C_m(x) / C_{ss}(x) \quad (2)$$

where $C_m(x)$ is the concentration of a specific metal (x) in tissue of species and $C_{ss}(x)$ the concentration measured in surface sediment for the same metal.

2.3.3 Statistical analysis

Environmental data is frequently characterised by exceptionally high values that deviate widely from the main body of data, thus data transformation may not always help to achieve a normal distribution and can lead to less conservative conclusions. Statistical analyses were carried out on transformed data where distributions were skewed, and if there was intractable heterogeneity, the results are discussed with appropriate reservations.

One way ANOVA and Tukey HSD post-hoc tests were applied to test for differences of chemical content in surface sediment between estuaries using Statistica (version 12, Statsoft Inc). Spearman rank correlation coefficient (r) was performed to examine the relationship between chemical parameters in sediment and biota using Statistica (version 12, Statsoft Inc). PERMANOVA was run on the entire set of data for metal content in surface sediments and species, to examine the significance of the variation between estuaries as well as among species (Primer 6, Primer-E ltd). Multivariate analysis such as correlation matrix analysis, is used to establish correlations among the various types of metals. Principal component analysis (PCA) is used to simplify the data and make it easier to identify the factors that explain most of the variance in the data. Sources

of metals were examined using PCA plots of areas within urban estuaries such as wetland (W), outlet (O), middle harbour (MH), outer side of Waimapu (B), outer side of Waikareao, Sulphur point (SP), and the wastewater treatment plant (WWTP) as shown in Fig. 2.1. Rural estuaries were not incorporated in this analysis as the number of sites and replicates were too small to permit robust statistical analyses. PCA has been used within species groups to visualise and compare the accumulation of metals in tissues between areas within estuaries. PCA has been proven to be a useful statistical tool that can be used to identify correlations and pattern in complex data sets, and hence suggest potential sources of contamination (Looi *et al.*, 2013).

2.4 Results

2.4.1 Trace element concentration in surface sediment

Trace element data from Rangataua, Waimapu, Waikareao, Tuapiro and Ohiwa in surface sediment showed differences among locations as demonstrated with the variability of EF and nested results above SQG safety guidelines (Table 2.3). The differences in metal content for different estuaries were statistically significant in many instances as derived by PERMANOVA ($P < 0.05$; Table 2.3).

Rangataua was the estuary characterised by the highest content of P and Ni. The calculation of EF showed no enrichment for V and Cr, minor enrichment for B, Ni, Cu, As and U, moderate enrichment for Pb, high enrichment for Zn and P, and very high enrichment for Cd (Table 2.3).

Waimapu had the highest content of Al and U while the lowest values reported for Cr and Zn. EF evaluation showed no enrichment for V, Cu, Cr and Ni, minor enrichment for B, As and U, moderate enrichment for Pb, and high enrichment for P, Zn and very high enrichment for Cd (Table 2.3).

Waikareao had the highest content of Cu and Pb and the lowest value reported for V. The EF for this estuary showed no enrichment for Cr, V, As and U, minor enrichment for B, Ni, Cu, moderately high enrichment for Pb, and severe enrichment for P, Zn and Cd (Table 2.3).

Tuapiro had the highest concentrations of B, V, Cr, Zn, As and Cd and the lowest concentration reported for Cu. EF showed no enrichment for Cu, Cr and U, minor enrichment for B, Ni, As and Pb, severe enrichment for P and Zn, and very severe enrichment for Cd (Table 2.3).

Ohiwa Harbour had the lowest concentrations for the B, Al, P, Ni, As, Cd, Pb and U, consistent with the absence of urban and industrial pollution in this harbour. EF calculation supports this interpretation, indicating no enrichment for B, U, V, Cr, Ni and Cu, minor enrichment for As and Pb. The exception to this overall trend was a moderate-severe enrichment for P and Cd and severe enrichment for Zn (Table 2.3). Given that Zn, P and Cd are all potentially fertilizer-derived elements; it is likely that these are the most important trace element contaminants in this rural setting. Overall, the average metal content of sediments was exceeded ISQG-low trigger values for Ni and Zn in Rangataua, Zn and Cd in Tuapiro, and Zn in Waimapu.

Table 2.3. Trace element abundance (mg/kg dry weight) in surface sediment in Rangataua, Waimapu, Waikareao, Tuapiro and Ohiwa estuaries; * ANOVA statistically significant $p < 0.05$, *I,II : Matching symbols denotes estuaries that are significantly different with each other (the superscript was used to highlight the small number of significant comparisons). NS: Non-significant. Sampling location as referred in Fig 2.1. ISQG-LOW: Interim sediment quality guideline- Low (ANZECC., 2000). ISQG-HIGH: Interim sediment quality guideline- High (ANZECC., 2000)

| Site | Elemental Concentration (mg/kg dry weight) | | | | | | | | | | | |
|-----------|--|---------------------|--------------------|---------------------|------------------|------------------|------------------|-------------------|-----------------|-----------------|------------------|-----------------|
| | B | Al | P | V | Cr | Ni | Cu | Zn | As | Cd | Pb | U |
| Rangataua | 56 ^{NS} | 14155 ^{NS} | 2001* | 20 ^{NS} | 7 ^{NS} | 37* | 16 ^{NS} | 289 ^{NS} | 6 ^{NS} | 2 ^{NS} | 14 ^{NS} | 1 ^{NS} |
| se | 11 | 2700 | 326 | 4 | 1 | 10 | 4 | 78 | 1 | 0 | 3 | 0 |
| EF | 3 | 1 | 18 | 1 | 0 | 3 | 2 | 20 | 3 | 36 | 5 | 2 |
| Waimapu | 46 ^{NS} | 17020* | 1548 ^{NS} | 16* ^I | 6 ^{NS} | 13 ^{NS} | 13 ^{NS} | 150 ^{NS} | 5 ^{NS} | 1 ^{NS} | 13 ^{NS} | 1.4* |
| se | 4 | 2763 | 300 | 1 | 1 | 2 | 2 | 29 | 0 | 0 | 2 | 0 |
| EF | 3 | 1 | 13 | 1 | 0 | 1 | 2 | 9 | 2 | 12 | 4 | 2 |
| Waikareao | 41 ^{NS} | 13595 ^{NS} | 1505 ^{NS} | 15* ^{II} | 7 ^{NS} | 21 ^{NS} | 17 ^{NS} | 292 ^{NS} | 5 ^{NS} | 1 ^{NS} | 22 ^{NS} | 1 ^{NS} |
| se | 6 | 1705 | 293 | 3 | 2 | 7 | 9 | 147 | 1 | 1 | 9 | 0 |
| EF | 2 | 1 | 12 | 1 | 0 | 2 | 2 | 17 | 2 | 20 | 6 | 1 |
| Tuapiro | 58 ^{NS} | 14236 ^{NS} | 1402 ^{NS} | 30* ^{I,II} | 10 ^{NS} | 18 ^{NS} | 10 ^{NS} | 389 ^{NS} | 7 ^{NS} | 3 ^{NS} | 12 ^{NS} | 1 ^{NS} |
| se | 11 | 2111 | 328 | 1 | 1 | 7 | 1 | 271 | 2 | 2 | 2 | 0 |
| EF | 3 | 1 | 11 | 1 | 1 | 2 | 1 | 23 | 3 | 48 | 3 | 1 |
| Ohiwa | 24 ^{NS} | 9597* | 944* | 22 ^{NS} | 8 ^{NS} | 8* | 10 ^{NS} | 194 ^{NS} | 4 ^{NS} | 0 ^{NS} | 10 ^{NS} | 0.6* |
| se | 8 | 2031 | 303 | 3 | 1 | 2 | 2 | 61 | 1 | 0 | 1 | 0 |
| EF | 1 | 1 | 8 | 1 | 0 | 1 | 1 | 12 | 2 | 7 | 3 | 1 |
| ISQG-LOW | | | | | 80 | 21 | 65 | 200 | 20 | 2 | 50 | |
| ISQG-HIGH | | | | | 370 | 52 | 270 | 410 | 70 | 10 | 220 | |

PCA analysis plots of data from the surface sediment within urban estuaries showed that PC1 and PC2 explained 77%, 73% and 89% of total variation in Rangataua, Waimapu and Waikareao respectively (Fig. 2.2). In Rangataua (Fig. 2.2A), all areas of the sub-estuary were correlated with metal accumulation. The stormwater drain had higher concentrations of P and Fe than other areas in this estuary. The outlet and middle harbour had an association with most metals while one site, around the WWTP, was associated mostly with Cd and Zn. In Waimapu (Fig. 2.2B), a clear contrast was observed between two groups: the wetland and the outlet which were associated with an accumulation of Al, U, As, Fe, Pb and P, while the concentration of metals was lower for the middle area of the sub-estuary and the outer part of the estuary. Samples collected within the wetland showed higher accumulation of metals than samples collected near the outlet. In Waikareao (Fig. 2.2C), most metals were accumulated near the storm water drain (Ni, Al, U, Zn, Pb, Cu and Cd) and the Sulphur Point area (P, As, V, Fe and Cr).

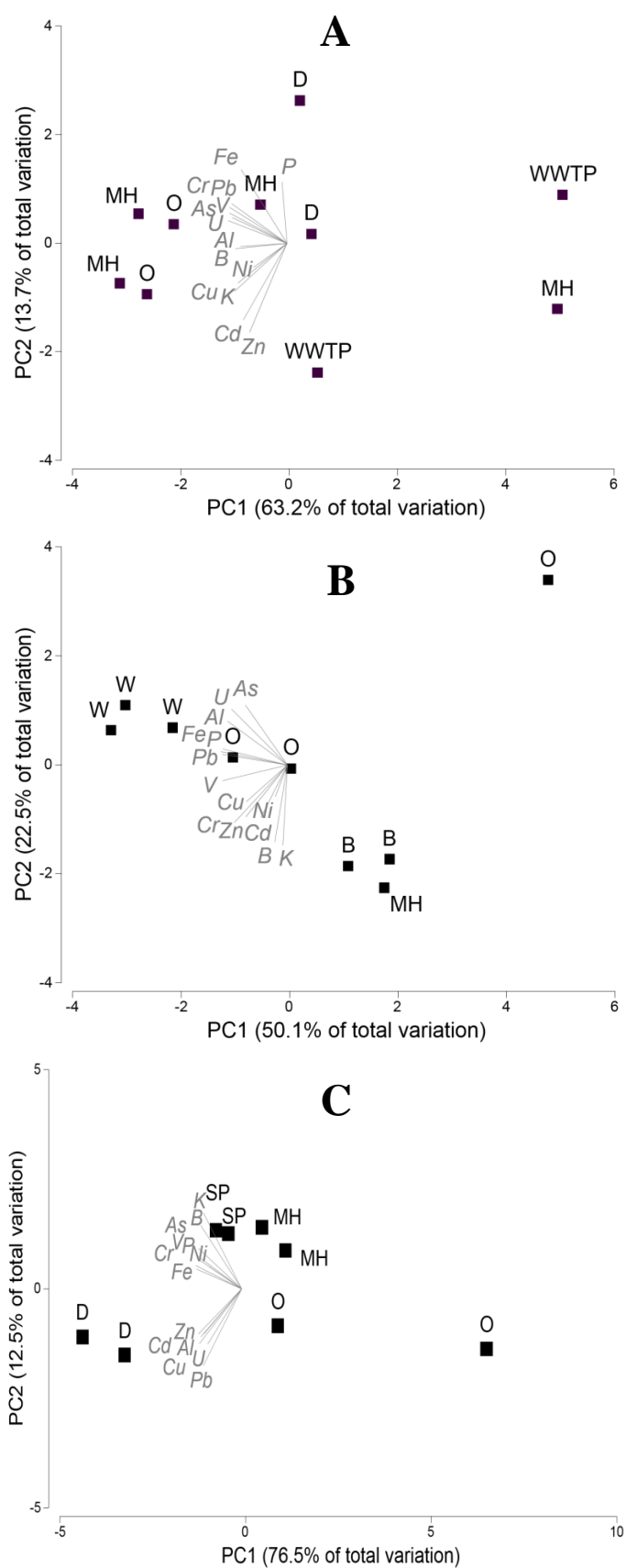


Fig. 2.2. Ordination plots of the primary component analysis of metal concentrations in surface sediment of Rangataua (A), Waimapu (B) and Waikareao (C). MH: middle harbour, O: outlet river, D: stormwater drain, WWTP: wastewater treatment plant, W: wetland, B: bridge, SP: sulphur point.

2.4.2 Trace element concentrations in biota

Zn, Cd and Cu concentrations were generally higher in invertebrates, except for B with higher concentration in seagrass, and Pb and Al with higher concentration in surface sediment (Fig. 2.3). For each figure, significance has been tested with PERMANOVA examining two factors and their mutual interactions: 'Estuaries' and 'Species'. Boron (B) concentrations indicated a significant variability among estuaries (PERMANOVA; $P < 0.05$) and species (PERMANOVA; $P < 0.01$). However, the combined interaction was not significant indicating a consistent relationship within the estuary and species factors. Boron was mainly concentrated in seagrass with higher concentration found in the Tuapiro estuary.

Sediment, sea grass, sea lettuce and mud snails exhibited the same pattern with higher concentrations of B found in Tuapiro, Rangataua, Waimapu, Waikareao and Ohiwa respectively (Fig. 2.3). Most differences in the Cu content were due to differences among species (PERMANOVA; $P < 0.01$) rather than location (PERMANOVA; $P > 0.05$).

Sediment, seagrass and sea lettuce showed similar concentrations of Cu with higher values in Rangataua and Waikareao, and lowest in Tuapiro. Mud snails and cockles had significantly higher concentrations of Cu than other species, with the maximum concentration found in Rangataua cockles. Variation of Zn and Cd among species and estuaries were identical and statistically significant for the two factors tested. Zn and Cd concentrations in sediment were higher in Tuapiro. However, Zn and Cd concentrations were even higher in Rangataua estuary for all species examined. Seagrass, sea lettuce and cockle showed similar variation among estuaries, with higher concentrations found in Rangataua and Waikareao.

Mud snails were more contaminated at Rangataua. Variation in Pb among estuaries was not significant. However, significant differences were recorded between surface sediment and biota, with higher concentrations found in sediment (Fig. 2.3). For all indicators, Pb was more concentrated in Waikareao. Variation of Al was similar to Pb, with no significant differences identified among estuaries. The concentration of Al in surface sediment was significantly higher than in biota, with mud snails containing significantly higher concentrations Al than both sea grass and sea lettuce.

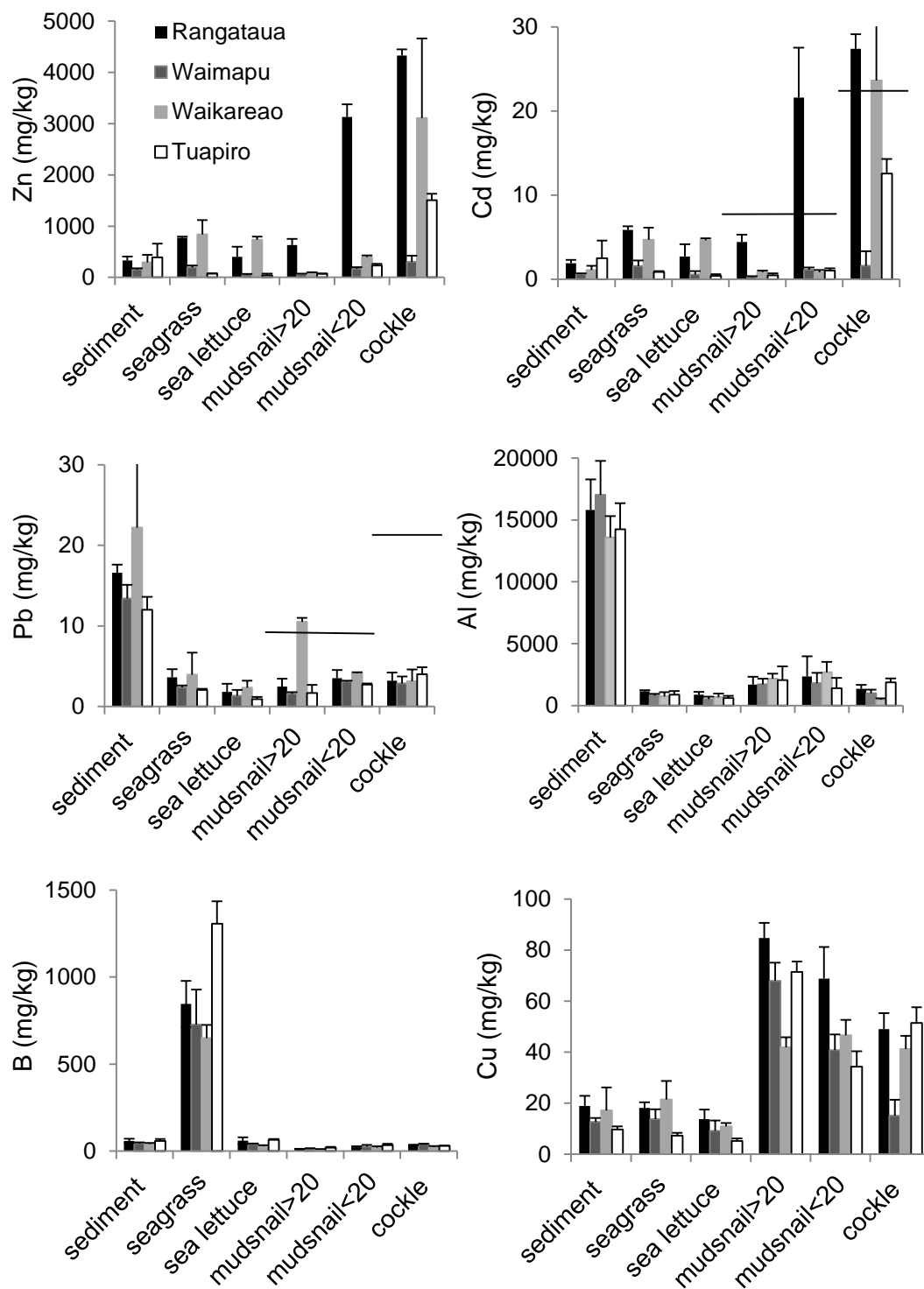


Fig. 2.3. Cd, Zn, Pb, Al, Cu and B content in surface sediment, seagrass, sea lettuce, adult mud snail (>20 mm), juvenile mud snail (<20 mm) and cockle in Rangataua, Waimapu, Waikareao and Tuapiro estuaries. The black horizontal line indicates the safety guideline threshold for seafood consumption after species specific conversion to dry weight (ANZECC 2000).

The correlation coefficients between metal content found in biota (seagrass, sea lettuce, small and large mud snails and cockle) and ambient concentration in sediment are listed in Table 2.4. Strong positive correlations were found between sediment and large mud snails for Pb and As. Pb also revealed a statistically significant positive correlation between the sediment and seagrass.

Table 2.4. The Spearman rank correlation coefficients between metal concentrations in sediment and biota. Bolding indicates statistically significant correlations $p < 0.05$. mudsnail > 20: mudsnail larger than 20 mm; mudsnail < 20: mudsnail smaller than 20 mm.

| | Surface Sediment | | | | |
|----|------------------|-------------|---------------|---------------|--------|
| | seagrass | sea lettuce | mudsnail > 20 | mudsnail < 20 | cockle |
| B | 0.09 | 0.20 | 0.45 | -0.03 | 0.60 |
| Al | 0.41 | 0.37 | 0.62 | 0.26 | 0.20 |
| P | 0.07 | 0.22 | -0.20 | -0.37 | 0.40 |
| V | 0.45 | -0.03 | 0.22 | -0.03 | 0.40 |
| Cr | 0.24 | 0.15 | 0.30 | 0.43 | -0.40 |
| Ni | -0.37 | -0.03 | 0.45 | -0.43 | 0.60 |
| Cu | 0.08 | -0.08 | 0.54 | 0.03 | 0.00 |
| Zn | 0.04 | -0.08 | 0.53 | 0.09 | 0.80 |
| As | -0.02 | 0.17 | 0.70 | 0.20 | 0.40 |
| Cd | 0.03 | -0.17 | 0.44 | 0.26 | 0.20 |
| Pb | 0.73 | 0.42 | 0.76 | 0.26 | 0.40 |
| U | 0.22 | 0.58 | 0.53 | 0.77 | 0.40 |

PCA including sediment and species discriminated metal content into three distinct groups, with the first two PCA explaining 65% of total variation (Fig. 2.4). The first group combined all surface sediment samples with V, Fe, Al and Pb. The second group clustered sea lettuce, sea grass and mud snails together for a wide range of metals at various concentrations. The last group associated all cockle samples with apparent accumulation preferences for Ni, Cu, P, Zn and As. The Bio-indicator Factor (BF) for a specific metal showed no major change with location except for cockle (Table 2.5). However, a large degree of variation in BF values was observed for different trace metals within the same species, indicating either the affinity of species to accumulate a specific trace element or a degree of uniformity in the underlying mechanisms of trace element accumulation by these

species. For example, elemental concentration in seaweed will be associated with suspended particles while mud snails will be more representative of the elemental composition found on the top of the sediment.

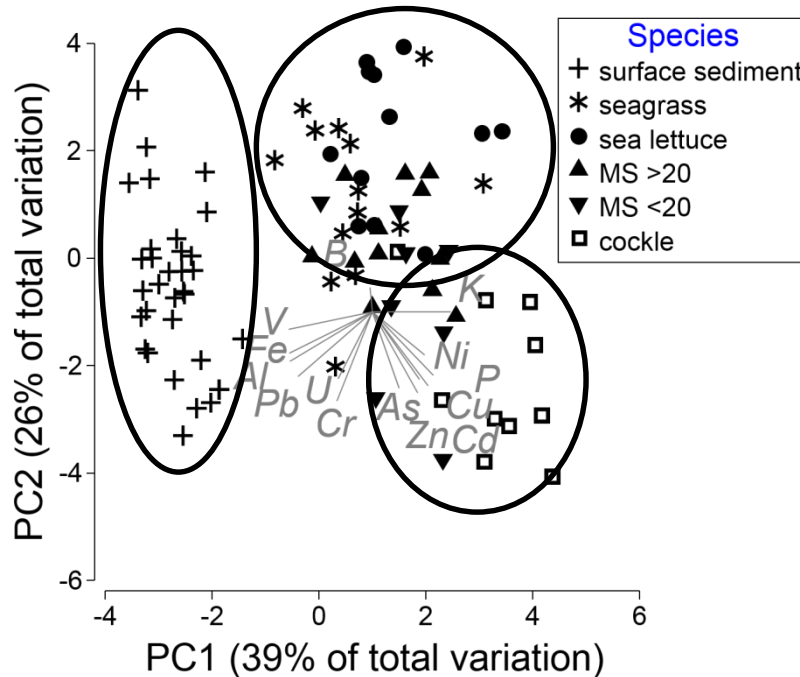


Fig. 2.4. Ordination plots of the primary component analysis of the metal concentrations in all indicators combined (MS>20: mud snails larger than 20mm, MS<20: mud snails smaller than 20mm).

Seagrass showed a higher BF in decreasing order of magnitude for B, Cd, Zn, Ni, P, Cu and U. A smaller BF was recorded for Al, V, Cr, As and Pb. PCA in seagrass (Fig. 2.5A) accounted for 67% of the total variance. The accumulation of most trace elements in seagrass blades appeared to be associated mainly with their proximity to point source discharges, namely the storm water drain, outlets of rivers and the WWTP. Trace element concentration in tissues clearly decreasing with distance from pollution source in urban estuaries.

Ulva generally showed a lower BF for most metals than seagrass, except for Al, Cr and As. The PCA on *Ulva* explained 63% of the total variance (Fig. 2.5B). Sulphur Point was associated with an increase of As, P and K. The stormwater drain and Rangataua outlet was associated with an increase mainly of

Zn, Cd and U. Outlets of the rivers were associated with most metals, particularly Cr, Cu, Pb, Fe and Al for Waimapu outlet.

Amphibola showed a high BF for Zn, Cd, Cu, P, Ni and As. For all sites studied, smaller mud snails (<20 mm) had higher BF for B, P, Cr, Ni, Zn Cd, Pb and U when compared to adults. Cu was the only exception, with higher content recorded in adults. PC1 and PC2 in the PCA for adult mud snails explained 59% of total variation (Fig. 2.5C). Mud snails exhibiting the most concentrated levels of the trace element group comprising Al, V, Cr, Fe, U, Cd and Zn, were associated with stormwater drains and river outlets. Tuapiro samples had higher concentrations of As, B, K and P, and the WWTP area was associated with higher concentrations of Cu. The first two principal components for juvenile mud snails (Fig. 2.5D) explained 77% of the total variation. Most metals (Cu, U, Pb, Zn, Cd, Fe, As, V and Al) were associated with outlets and storm water drains while P, Ni, Cr, K and B were associated with the middle harbour of Waimapu. There was a close association of trace element concentrations in juvenile mud snails for all sites in Rangataua estuary, with higher content in decreasing order found in the outlet, drain and middle harbour. The WWTP area was associated with increases of Cu.

The BF of cockles was high for As, P, Ni, Zn, Cd and Cu. PCA of cockles accounted for 69% of the total variance (Fig. 2.5E) with three groups of metals identified that corresponded with distinct locations. The bridge area of Waimapu was associated with Pb, Al, Fe, Cr, Ni and B. Ohiwa, Tuapiro and Sulphur Point (land) were associated with Cu, Cd, Zn, U and V. Finally, a distinct group was observed for As, K, and P associated with the Sulphur Point (ocean).

Table 2.5. Bioaccumulation factor (BF) in organisms in Bay of Plenty estuaries. $BF = C_m(x) / C_{ss}(x)$ where $C_m(x)$ is the concentration of a specific metal (x) in tissue of species and $C_{ss}(x)$ the concentration measured in surface sediment for the same metal. Detailed locations are given in Fig. 2.1.

| | | B | Al | P | V | Cr | Ni | Cu | Zn | As | Cd | Pb | U |
|--------------------|-------|------|-----|-----|-----|-----|-----|-----|------|-----|-------|-----|-----|
| Mudsnails | | | | | | | | | | | | | |
| Rangataua | >20mm | 0.2 | 0.1 | 1.4 | 0.1 | 0.4 | 0.9 | 4.5 | 1.9 | 1.0 | 2.3 | 0.1 | 0.5 |
| | <20mm | 0.4 | 0.1 | 1.9 | 0.2 | 0.7 | 1.9 | 3.6 | 9.5 | 0.9 | 11.4 | 0.2 | 1.3 |
| Waimapu | >20mm | 0.3 | 0.1 | 2.3 | 0.1 | 0.4 | 1.2 | 5.4 | 0.4 | 1.2 | 0.5 | 0.1 | 0.3 |
| | <20mm | 0.6 | 0.1 | 2.4 | 0.1 | 0.7 | 3.8 | 3.2 | 1.1 | 0.7 | 1.9 | 0.2 | 0.3 |
| Tuapiro | >20mm | 0.3 | 0.1 | 2.6 | 0.1 | 0.3 | 1.6 | 7.4 | 0.2 | 1.4 | 0.2 | 0.1 | 0.2 |
| | <20mm | 0.6 | 0.1 | 2.7 | 0.0 | 0.6 | 3.6 | 3.5 | 0.6 | 0.7 | 0.4 | 0.2 | 0.3 |
| Seagrass | | | | | | | | | | | | | |
| Rangataua | | 14.3 | 0.1 | 0.8 | 0.3 | 0.3 | 0.6 | 1.0 | 2.3 | 0.6 | 3.1 | 0.2 | 1.2 |
| Waimapu | | 15.8 | 0.0 | 1.5 | 0.3 | 0.6 | 3.6 | 1.1 | 1.3 | 0.4 | 2.6 | 0.2 | 0.9 |
| Waikareao | | 15.8 | 0.1 | 1.6 | 0.3 | 0.5 | 1.2 | 1.3 | 2.9 | 0.6 | 4.3 | 0.2 | 1.0 |
| Tuapiro | | 22.7 | 0.1 | 1.1 | 0.2 | 0.3 | 1.5 | 0.7 | 0.2 | 0.2 | 0.4 | 0.2 | 1.3 |
| Sea lettuce | | | | | | | | | | | | | |
| Rangataua | | 0.1 | 0.8 | 0.6 | 0.3 | 1.8 | 4.8 | 0.8 | 0.5 | 0.9 | 0.5 | 0.5 | 0.1 |
| Waimapu | | 0.1 | 0.6 | 0.5 | 0.3 | 0.8 | 0.7 | 0.7 | 0.3 | 2.4 | 0.4 | 0.6 | 0.1 |
| Waikareao | | 0.0 | 0.9 | 0.6 | 0.6 | 0.7 | 0.8 | 0.5 | 0.9 | 1.5 | 1.0 | 0.6 | 0.2 |
| Cockles | | | | | | | | | | | | | |
| Rangataua | | 0.6 | 0.1 | 2.8 | 0.0 | 0.8 | 2.2 | 2.6 | 13.1 | 2.8 | 14.4 | 0.2 | 1.1 |
| Bureta land | | 0.7 | 0.0 | 3.8 | 0.0 | 1.0 | 8.3 | 5.9 | 43.0 | 5.0 | 173.1 | 0.4 | 1.1 |
| Bureta ocean | | 0.3 | 0.0 | 5.0 | 0.0 | 0.6 | 1.7 | 3.0 | 5.5 | 4.3 | 9.4 | 0.1 | 0.6 |
| Ohiwa | | 0.9 | 0.1 | 6.2 | 0.1 | 0.8 | 9.9 | 5.6 | 11.1 | 4.4 | 45.0 | 0.2 | 1.2 |

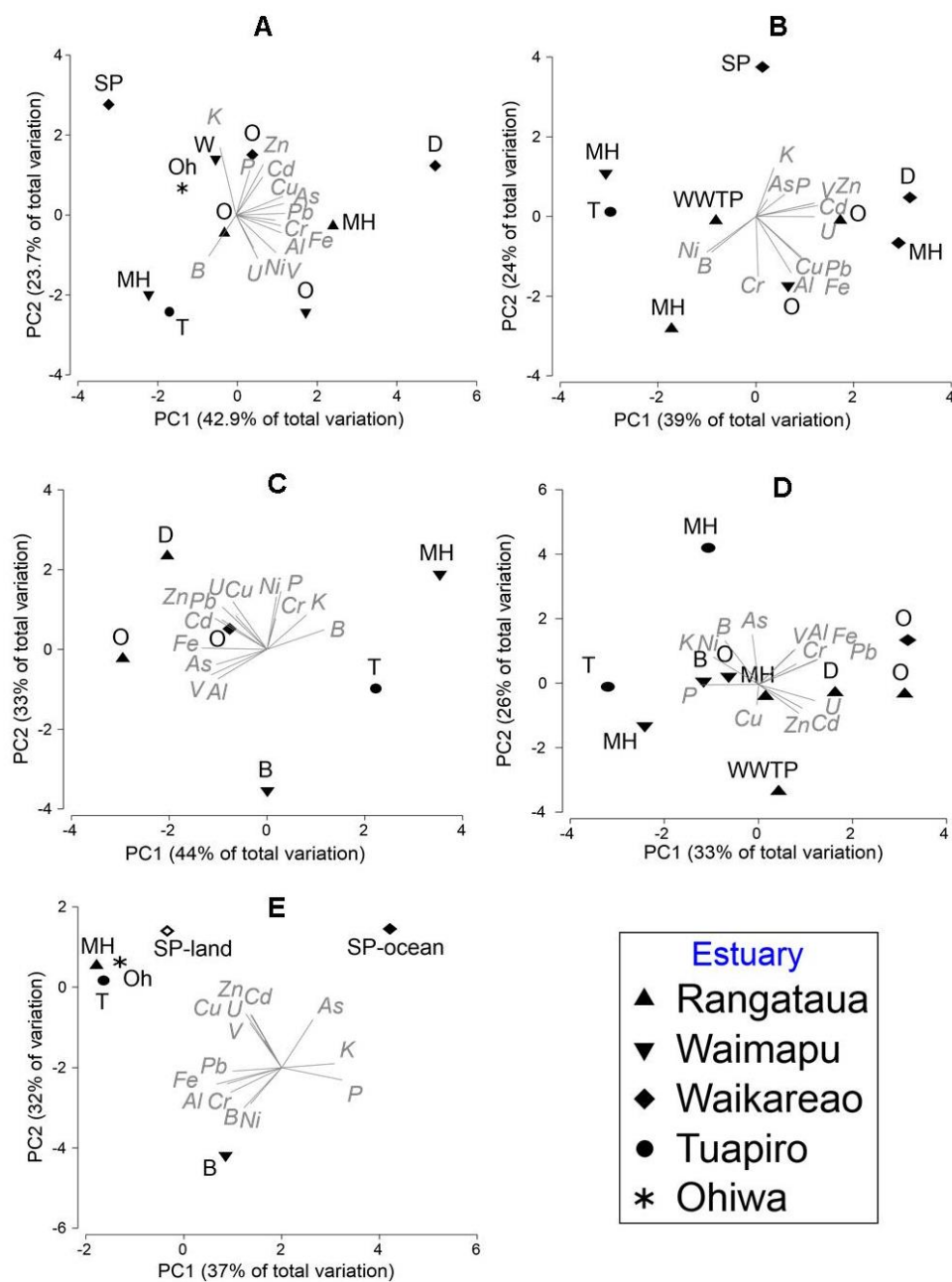


Fig. 2.5. Ordination plots of the primary component analysis of the metal concentrations in seagrass (A), sea lettuce (B), mud snails >20 mm (C), mud snails <20 mm (D) and cockles (E); MH: middle harbour, O: outlet river, D: stormwater drain, WWTP: wastewater treatment plant, W: wetland area, B: bridge, SP: sulphur point.

2.4.3 Trace metal distribution in urban and rural environments

A comparison of trace element accumulation in urban, rural and other locations in surface sediment shows that B, Al, P, Ni, Cu, Pb, P and U are higher in urban settings while V, Cr, Zn, Cd and Fe are typically more enriched in rural areas (Table 2.6). As and K showed no difference between urban and rural settings. Analysis of variance showed a significant result for P ($F_{(1,16)}=4.89$; $P<0.05$) and V ($F_{(1,16)}=5.25$; $P<0.05$).

In seagrass, B was the only trace element found in higher concentration in rural environments, while V was found to be approximately the same in urban and rural settings. All other elements were more concentrated in the urban environment. Differences between urban and rural environments were only significant for B ($F_{(1,13)}=10.26$; $P<0.05$). However, the ecological meaning underlying this statistical observation is unclear.

For mud snails, animals from the urban environment had higher Al, Ni, Cr, Zn, Cd, Pb, U and Fe than those from rural locations. In the rural environment, we found higher B, P, V, Cu, As and K (Table 2.6). One way ANOVA showed statistically significant differences between environments for K ($F_{(1,11)}=7.87$; $P<0.05$) and U ($F_{(1,11)}=5.56$; $P<0.05$). Finally, cockles collected from the urban environment had more B, P, Ni, Zn, As, Cd, K, Pb and U in their tissues, while those from the rural environment showed greater concentration of Al, V, Cr, Cu, and Fe. The results were statistically significant for P ($F_{(1,8)}=8$; $P<0.05$), Fe ($F_{(1,8)}=28.3$; $P<0.01$) and V ($F_{(1,8)}=203.4$; $P<0.001$).

Table 2.6. Elemental concentration in urban and rural habitat in Bay of Plenty estuaries and other New Zealand and international estuaries. TF: total fraction of sediment analysed, Con: contaminated, Uncon: uncontaminated

| | Elental concentration (mg/kg dry weight) | | | | | | | | | | | | | | Reference |
|-------------------------------------|--|-------|------|------|----|------|----------|-------|---------|----------|------|-----------|-------|---|-----------------------------|
| | B | Al | P | K | V | Cr | Fe | Ni | Cu | Zn | As | Cd | Pb | U | |
| Surface sediment (<64 µm) | | | | | | | | | | | | | | | |
| Urban, NZ | 49 | 15474 | 1770 | 1387 | 18 | 7 | 12188 | 25 | 16 | 257 | 6 | 1 | 17 | 1 | This study |
| Rural, NZ | 41 | 11916 | 1173 | 1393 | 26 | 9 | 12234 | 13 | 10 | 292 | 6 | 1 | 11 | 1 | This study |
| Rangataua, NZ - TF | | | | | | 2 | | 1 | 2 | 19 | 3 | 0 | 3 | | Park., 2012 |
| Waimapu, NZ- TF | | | | | | 6 | | 2 | 6 | 53 | 4 | 0 | 9 | | Park., 2012 |
| Waikareao, NZ- TF | | | | | | 4 | | 2 | 2 | 87 | 4 | 0 | 6 | | Park., 2012 |
| Ohiwa, NZ- TF | | | | | | 8 | | 6 | 2 | 37 | 6 | 0 | 7 | | Park., 2012 |
| Whau upper, Auckland. Con | | | | | | | | | 35 | 270 | | | 68 | | Mills et al., 2012 |
| Urban Christchurch, NZ - TF | | | | | | 7 | 15000 | 2 | 4 | 36 | 1 | | 2 | | Marsden et al., 2014 |
| Rural Christchurch, NZ - TF | | | | | | 7 | 16500 | 2 | 4 | 33 | 2 | | 2 | | Marsden et al., 2014 |
| Urban Christchurch, NZ - TF | | | | | | 15 | | 9.1 | 7.4 | 88 | 3 | 0 | 16 | | Bolton-Richie & Lees., 2012 |
| Texas, USA. Uncon | | | | | | | 9148 | | 12 | 33 | 6 | | 5 | | Whelan et al., 2005 |
| Greece. Con | 217 | 3050 | | | 27 | 34 | | 10 | | | 2 | | | 0 | Malea and Kevrekidis., 2013 |
| Zostera marina | | | | | | | | | | | | | | | |
| Urban, NZ | 731 | 882 | 2222 | 9267 | 5 | 3 | 3416 | 33 | 17 | 553 | 3 | 4 | 3 | 1 | This study |
| Rural, NZ | 1330 | 740 | 1625 | 4168 | 5 | 2 | 1185 | 29 | 9 | 309 | 2 | 3 | 2 | 1 | This study |
| Texas, USA. Uncon | | | | | | | 457 | | 10 | 20 | 1 | | 1 | | Whelan et al., 2005 |
| Greece. Con | 1438 | 731 | | | 7 | 12 | | 2 | | | 2 | | | 0 | Malea and Kevrekidis., 2013 |
| Ulva lactuca | | | | | | | | | | | | | | | |
| Urban, NZ | 43 | 690 | 1257 | 5449 | 2 | 3 | 857 | 54 | 11 | 397 | 4 | 3 | 2 | 0 | This study |
| Urban, NZ | | 1600 | | | | 3 | 1200 | 3 | 16 | 18 | | | 2 | | Barr., 2007 |
| Rural, NZ | | 250 | | | | 1 | 200 | 1 | 16 | 12 | | | 1 | | Barr., 2007 |
| Argentina. Uncon | 52 | | 1910 | | 4 | 1 | 201 | 1 | 2 | 22 | 5 | 1 | 1 | | Perez et al., 2007 |
| Argentina. Con | 58 | | 1750 | | 6 | 1 | 532 | 1 | 3 | 17 | 6 | 0 | 1 | | Perez et al., 2007 |
| Otago, NZ, Con | | | | | | | | | 0.9-3.5 | 5.6-26.8 | | | | | Brown et al., 1999 |
| Hong Kong, China. Uncon | | | | | | | 50-380 | 4-10 | 5-18 | 8-38 | | 0.67-1.20 | 4-13 | | Ho., 1990 |
| Hong kong, China. Con | | | | | | | 690-3960 | 12-48 | 34-132 | 31-110 | | 0.46-0.88 | 10-54 | | Ho., 1990 |
| Amphibola crenata | | | | | | | | | | | | | | | |
| Urban, NZ | 14 | 1758 | 3232 | 4964 | 2 | 3 | 2024 | 27 | 74 | 350 | 6 | 2 | 3 | 0 | This study |
| Rural, NZ | 15 | 1594 | 3858 | 6322 | 3 | 3 | 1333 | 27 | 77 | 96 | 8 | 1 | 2 | 0 | This study |
| Austrovenus stuchburyi | | | | | | | | | | | | | | | |
| Urban, NZ | 30 | 810 | 7032 | 7412 | 0 | 6 | 426 | 85 | 40 | 3057 | 24 | 22 | 3 | 1 | This study |
| Rural, NZ | 25 | 1455 | 5807 | 5686 | 3 | 7 | 1325 | 66 | 54 | 1941 | 20 | 16 | 3 | 1 | This study |
| Urban, Otago, NZ | | | | | | 1-44 | | 5-35 | 3-60 | 40-118 | | | | | Peake et al., 2006 |
| Urban, Cristchurch, NZ | | | | | | 3 | 556 | 2.4 | 7 | 51.9 | 24 | 0.2 | 1 | | Marsden et al., 2014 |
| Rural, Cristchurch, NZ | | | | | | 1 | 485 | 2 | 9 | | 98.7 | 0.4 | 0.5 | | Marsden et al., 2014 |
| Otumoetai, NZ | | | | | | 3.6 | 122 | | 13.3 | 111 | 35 | 0.6 | 0.3 | | McIntosh., 1999 |
| Ohiwa, NZ | | | | | | 3.9 | 288 | | 8.9 | 838 | 30 | 0.6 | 0.3 | | McIntosh., 1999 |

2.5 Discussion

2.5.1 Pollution risks, sources and fate of metals in surface sediment

Increases in metal contaminants in estuaries are one of the primary causes of ecological degradation on a global basis (Zhou *et al.*, 2008; Garcia-Garcia *et al.*, 2012). Metals have potential toxic consequences for intertidal biota such as mud snails, fishes, and humans when consuming such contaminated seafood (Spry & Wiener, 1991; Ryan & Hightower, 1994). An accurate understanding of the metal content in sediment and associated biota is needed to estimate the degree of pollution in an ecosystem and the associated risks linked to recreational and commercial activities that involve consumption of food. Such information will directly support environmental authorities charged with remediation and mitigation of pollution sources. With this in mind, trace metals have been measured in a range of stressed estuaries where anthropogenic inputs are present. Surface sediment has been used as a tracer for elemental accumulation and potential sources of pollution including their fates in urban and rural areas. In this paper, we started our interpretation by comparing different estuaries regarding their metal profiles and assessed this via New Zealand guidelines and international benchmarks as reported in the literature. By considering and comparing sub-areas within each estuary, as presented in PCA plots, we were able to refine our observations to a smaller scale and thereby raise hypotheses as to the range of sources and bioaccumulation pathways that may be operating across differing ecosystems influenced by differing types of anthropogenic inputs.

Tuapiro, Waikareao, Waimapu and Rangataua showed levels of contamination above New Zealand ANZECC guidelines for Ni, Zn and Cd, while Ohiwa Harbour was the most pristine estuary. It was clear that each estuary, due to their respective locations and surrounding environments, displayed distinct

chemical signatures with Rangataua being the most affected, based on a higher EF for most metals. In Rangataua, the metal contamination was widespread in the estuary and was probably influenced by contaminated freshwater from the Waitao River and from the storm water drain (particularly with regard to P). It appeared that localised point sources such as a drain can influence the chemical budget of the entire estuary as shown by the severe enrichment factor found for P in Rangataua. Discharge of metals from the river was not the only source of metals in Rangataua, since higher concentrations were found in the middle estuary compared to the outlet area. In Waimapu, the accumulation of most metals occurred in the wetland area and the river outlet, and showed similar patterns of contamination to Rangataua. Metal concentrations in sediment gradually reduced from the wetland towards the outer side of the estuary. This observation confirmed the action of the wetland for trapping contaminants before they are released to other parts of the harbour. The 90% decrease of wetland area following European settlement in New Zealand (Ausseil *et al.*, 2007), together with the added release of contaminants into the environment, is therefore of great concern. With the reduction of wetland area, contaminants can be transmitted at greater distances from the contamination source, affecting organisms and food chains. In the Waikareao Estuary, similar metal accumulation was found around the stormwater drains, but little accumulation was detected around the stream outlet. However, the outer part of the estuary known as Sulphur Point, which is directly open to the ocean and associated with various industrial point source discharges, was characterised by accumulation of B, K, As, V, P, Fe and Cr. This finding was similar to the results of a study looking at trace metals enrichment from stormwater in the southern part of the Tauranga Harbour (McIntosh & Deely, 2001). The authors found an increase of As and Pb by 4 fold, Cr, Cd and Cu by 6

fold, and Zn by 23 fold when compared to a control stormwater drain. Murphy *et al.* (2015) reported Cu, Zn and Pb deposition in stormwater systems in Christchurch, New Zealand. These authors found a close association between metal deposition and rain events, with rainfall intensity, duration and depth having an influence on Cu, Zn and Pb loads in stormwater runoff, which varied with the type of the pollutant. High variability in Al, Ca, Cu, Fe, Mg, Pb and Zn loads from stormwater drains over short periods of time have also been measured in Sydney Estuary, Australia (Beck & Birch, 2014). These authors emphasised the role of the industrial origin of metal contamination which was significantly greater during higher levels of business activity.

While the land use was similar between Ohiwa and Tuapiro estuaries, Tuapiro showed greater accumulation of metals, arguably due to intensive agriculture with accumulation of Zn, Cd, As, B, V and Cr. The strong correlation between Zn and Cd suggested that they are associated with intensive use of fertilisers. For most estuaries, we found a gradient of decreasing trace element contamination with distance from the inferred source, except for the most heavily contaminated estuaries (e.g. Rangataua), which were characterised by a more widespread halo of contamination affecting the entire estuary.

The Bay of Plenty Regional Council reported trace metal concentrations in surface sediment in Tauranga and Ohiwa Harbour (Park, 2003; Park, 2014). The author found similar trace metal concentrations in Rangataua, Waimapu, Waikareao and Ohiwa estuaries. Previous research in Auckland showed important fluctuation in trace metal concentrations between sediment sieved at 500 μm and 64 μm especially for sites with low mud content (Mills *et al.*, 2012). Therefore, the results collected by the Bay of Plenty Regional Council cannot be directly

compared to the present study as the Regional Council analysed trace elements for the whole sample (total) while we analysed elemental concentration for the fine sediment fraction (<64µm). Cu, Zn and Pb has been analysed by the Auckland City Council for the fine sediment fraction at pristine and very polluted areas. Concentration of Cu and Pb in the present study were lower than a contaminated estuary (Whau upper) by 2 and 3 fold respectively; however Zn had similar concentrations between these two estuaries.

2.5.2 Metal accumulation in biota

We used a similar approach to examining metal contamination in biota, examining samples from a broad spatial range together with detailed examination within locations and across size ranges for certain organisms. This analysis indicated that seagrass, sea lettuce, mud snails and cockles displayed different tendencies to accumulate metals, resulting in a high level of complexity in the trace element distribution. New Zealand Safety guidelines for seafood consumption (ANZECC, 2000) were exceeded in some cases (Cd, Zn, Fig. 2.3) showing a potential threat to human health. Variability in contaminating metal loads within and among species related to the species' feeding method, rather than to the concentration of the metal in the surface sediment. In our study, the degree of metal accumulation in soft tissue did not follow the same order and spatial variations as in the associated sediments for most trace metals (as showed by weak Spearman rank correlation coefficients). Seagrass, sea lettuce and mud snails had similar degrees of variance with respect to their metal concentrations. For example, the high accumulation of B in seagrass contrasted with a low EF in sediment, probably due to direct assimilation from seawater as H_2BO_3 (Woods,

1994). Similar accumulation trends were observed between molluscs with generally higher concentrations of Cu, Zn and Cd. Other elements such as Al and Pb were highly concentrated in sediments but showed low concentrations in the examined biota. Generally in New Zealand, biological samples screened for safe human consumption have been well below safety guidelines for seafood consumption (Thomson *et al.*, 2008; Whyte, *et al.*, 2009). However as mentioned above, accumulation of metals in species not routinely monitored can reach toxic levels that will be amplified by the large amount of seafood consumed by indigenous people rather than the general public of New Zealand.

An explanation for elemental variability in biota may be the difference in the locations occupied by individual species. For example, we only found mud snails on the landward edge of harbours while cockles were present in more open spaces. As mentioned previously, the ambient sediment chemistry was different from one estuary to another, with some important intra-estuarine variance being evident, which may have resulted in certain elements being accumulated to greater degree with greater proximity to point sources.

The most contaminated biota was found near to sources of riverine inputs to the estuaries and stormwater drains. River inputs and human infrastructure such as stormwater outlets appear to be sources of pollution for biota. Other more localised sources of contamination affected the two mollusc species. Cockles were sampled at two sites within the Sulphur Point vicinity (Bureta-land separated by 300 m to Bureta-ocean). The difference in elemental concentration in cockle tissues between these two sites was profound (Table 2.4), showing the local importance of point source discharges in estuarine pollution as well as the high sensitivity of filter feeding species to differences in water chemistry. A previous

study using *Austrovenus stutchburyi* under laboratory conditions (Fukunaga & Anderson, 2011) found Pb content in tissue similar to this study, however Zn and Cu concentrations were 13 and 4 times higher, demonstrating that it concentrates toxic metals in the presence of differing mixtures of contaminants. Cr, Fe, Cu, Zn, Cd and Pb in cockle tissues collected in urban and rural estuaries were higher than the contaminants levels measured in 1999 demonstrating a general increase of trace elements with time (McIntosh., 1999). However, the concentration of As was lower in our present study showing a potential decrease in the use of As in most recent time.

As shown by its higher elemental concentration and BF, seagrass accumulated more metals than sea lettuce. Differences in metal concentration between seagrass and sea lettuce can be explained by their different physiology and mode of attachment which determines the pathway of metal uptake. Sea lettuce is a free floating macroalgae that can also be fixed to small dead shells and rock fragments by a fragile holdfast, while seagrass is an angiosperm having complex fine root systems providing a much more secure attachment to the sediment, hence largely immobile. Uptake of metals for seagrass and sea lettuce are from their blades; however, seagrass has an additional pathway of metal uptake via roots. Thus, seagrass is used as an indicator of metals in water and sediment. Sediment characteristics such as grain size, mineral composition, and organic matter content may also influence the overall biogeochemical environment of the root zone. Sea lettuce metal content therefore primarily reflects the uptake of dissolved metals from the water column (Phillips, 1990). Our research has demonstrated that macrophytes such as seagrass and sea lettuce have different affinities for specific metals shown by higher metals content for seagrass in the middle harbour of Rangataua than the outlet, and an opposite

observation for sea lettuce with higher content near the outlet. Macrophytes are at the bottom of the trophic web and metals can be potentially bio-accumulated to higher trophic species when consumed by herbivores such as mud snails and fishes (Croteau *et al.*, 2005; Fraser *et al.*, 2005; Terra *et al.*, 2008).

Adult and juvenile mud snails accumulated metals at different concentrations depending on their size, irrespective of the estuary. Juvenile mud snails had more metals than adults (except for Cu). This difference between life stage possibly corresponds to different diets, food selection or differential efficiency for excretion of waste. Juvenile invertebrates are known to be generally more sensitive to toxicity than adults (Ahsanullah & Florence, 1984; Green *et al.*, 1986) probably due to a more rapid accumulation of metals. Additionally, this difference in metal accumulation could be due to the recent deposition of enriched sediment identified by the presence of active metal source. Mud snails accumulated trace elements in elevated concentrations but it was not possible to estimate the potential toxicity effects of such trace metals as no research has been conducted on this species. From a health perspective, safety guidelines for human consumption may be exceeded for Cd, Pb and other metals not usually screened for Cu, Zn and Al as these were present in very elevated concentration.

2.5.3 Advances in biological, environmental and chemical monitoring

Environmental publications concerning metal contamination across a broad range of endemic species is relatively limited in New Zealand. This study has attempted to provide new information on the factors influencing trace element accumulation in native biota by focusing on common native estuarine species that also constitute food sources for local communities. While sediment has been proven a robust indicator for metal pollution (Audry *et al.*, 2004; Caeiro *et al.*,

2005), the outcome of such analyses appears to have somewhat reduced ecological relevance when benchmarking or comparing ecosystem health. Though surface sediment elemental composition was a good index for general environmental monitoring, it was not a reliable indicator of elemental accumulation in organisms. The fact that all species have unique physiological proprieties suggests that they will accumulate trace elements via different mechanisms. This combined with the fact that elemental speciation varies to a very large degree depending on the specific physiochemical conditions, means that the bioaccumulation of trace elements in estuarine systems is exceedingly complex. The speciation of metals in the environment can be a principal factor dictating the accumulation of metals in organisms. Hence given the lack of knowledge about how sedimentary chemistry (with regard to the contamination by metals) influences the bioaccumulative capacity of different species of estuarine organism, relying on sedimentary chemical profiles alone as an indicator of environmental health can be misleading.

Seagrass, sea lettuce and mud snails showed little variation in their BF for specific metals across a range of estuaries. Thus, these species may be useful indicators of chemical contamination between locations. However, cockles showed more variability in their BF, possibly explained by their filter feeding diet, with minimal uptake of trace elements from sediment. Therefore, cockles may be an additional indicator organism suitable for integrating water chemistry in monitoring programmes. The BF indicated which elements and species had the highest propensity to concentrate contaminating metals and hence can be used as a proxy when the chemical content of the pollution source is known. In this case, seagrass appeared to be very sensitive to B, Bi, Zn, Cd and U as indicated by their higher BF values. Ulva appeared to be a good indicator for Ni, Cu, As, Cd and Al.

Mud snails appeared to be a sensitive species for the detection of Cu, Zn, A, Cd, Ni, Cr and P and cockles for the dissolved concentration of B, P, Ni, Cu, Zn, As, Cd and U.

By comparing metal concentrations in species, complementary information can be assembled, such as the history of contaminants in a particular area (past pollution events) and patterns of metal speciation relative to the medium the pollutant is concentrated (dissolved and particulate phases in water, sediment). For example, by comparing metal content in seagrass (which is more representative of the metal content in sediment possibly through integration of historical contamination events via root uptake), with sea lettuce (that has a direct and more instantaneous association with water chemistry), arsenic was found more concentrated in the sea lettuce. This may be indicative of greater bioavailability of As in solution rather than sediment. Furthermore, cockles were five-fold more contaminated in As than sediments, which possibly indicates that arsenic is mainly present in the particulate and colloidal fraction of the water, and can be rapidly accumulated by water filter feeders. The association of As with ambient elements was however different among species. Those species assimilating elements mainly from water such as sea lettuce and cockle had a different accumulation profile (As, P, K, Zn, Cd, U) compared with sediment-dwelling organisms such as seagrass and mud snails (As, Cu, Pb). This could indicate the different origins of As, e.g. between anthropogenic sources such as paint, wood preservers, fertilisers and pesticides, which inevitably have different metal compositions and specific solubility in the water. The elemental composition measured in cockle and sea lettuce both showed enrichment in Cd and U, possibly originating from agricultural P fertiliser applications (Schipper, *et al.*, 2011).

Mud snails of different sizes showed different accumulation rates of metals. Differences in metabolic pathways during life history stages in mud snails may be responsible for this, but are poorly understood and did not allow further investigation in this study. However, it highlighted the significance of sampling invertebrates across a size range when doing environmental monitoring to understand the range of variation due to the size/age/physiological state of species hence providing more relevant information about the sensitivity of more vulnerable life history phases (Green, *et al.*, 1986). Additionally, elemental concentration in smaller mud snails could be used to identify the presence of recently introduced metal sources while larger mudsnails could integrate across a longer pollution history.

Land use practice analysis within catchments has been a useful tool for ecologists and environmentalists to indirectly assess the state of the environment and/or estimate the risk associated with potential contamination of connected ecosystems (Allan *et al.*, 1997; Quinn *et al.*, 1997; Snyder *et al.*, 2003). Precautions have to be taken however, when using land use information (only) as an environmental indicator. Firstly, a rural site cannot always be associated as a pristine site where industries are transforming and eroding the land; as agricultural activities dominated by the intensive use of pesticides and fertilisers are responsible for the leachate of many trace elements and toxic compounds (legacy effects). Some pesticides have been prohibited in other countries and are now proving to be dangerous and toxic for human populations (Nigg & Knaak, 2000; Abdollahi *et al.*, 2004; Cox & Sorgan, 2006; Dawson *et al.*, 2010). Secondly, adjacent catchments can sometimes be connected by surface water and thus be contaminated by external sources but will frequently be connected by estuarine groundwater discharge. Hence any examination of estuarine health should factor

in the management of adjacent catchments. While they had similar land uses, Ohiwa had lower elemental content (B, Al, P, N, Zn, Cd and Pb) than Tuapiro; therefore was significantly less polluted due to the prevalence of native forest localised in and around the catchment.

The results of this study provides an elemental baseline for surface sediment, seagrass, sea lettuce, mud snails and cockles in urban and rural estuaries of Northern New Zealand. This baseline can be used to compare data with international datasets associated with the health of the estuarine environment. A previous metal accumulation study from Christchurch, New Zealand, found that the Cr concentration in surface sediments was similar for urban and rural estuaries (Marsden *et al.*, 2014). Sedimentary Fe concentration was greater in Christchurch, while Ni, Cu, Zn, As and Pb concentration were higher in the Tauranga Harbour. These results were reflected in the tissues of cockles with higher concentration of Cr, Ni, Cu, Zn, Cd and Pb in Tauranga, while Christchurch had greater concentrations of Fe (only for urban) and As (only for rural). Al, As, B (only rural) and V concentrations measured in seagrass tissues from Tauranga Harbour were similar to a contaminated site found in Greece (Malea & Kevrekidis, 2013). However, Cr concentrations in Greece were greater than our study, while Tauranga Harbour had higher concentrations of Ni. Trace elements in *Ulva* were compared with a national urban baseline for New Zealand (Barr, 2007). Zn and Ni contents in *Ulva* tissues of Tauranga were greater than the national baseline, while smaller for Al, Cu and Fe. Sea lettuce tissue collected in Otago harbour had smaller Cu and Zn concentrations than those collected in the Tauranga Harbour showing trace metal enrichment in Tauranga Harbour water (Brown *et al.*, 1998). Fe, Ni, Zn and Cd content in sea lettuce collected in Tauranga Harbour had similar concentration than a contaminated estuary in Hong Kong while Pb content

in Tauranga was representative of a relatively uncontaminated environment (Ho., 1999).

We highlight the high levels of Cu, Ni, Cd and Zn in surface sediment and biota generated by natural and anthropocentric origins.

2.6 Conclusion

From this research, it was clear that most estuaries in the Bay of Plenty were affected by metal contamination as demonstrated by metal concentrations exceeding sediment trigger values (Ni, Cd and Zn). The calculation of EF and the comparison with other urban and rural estuaries reinforced this conclusion. The different degree of contamination in sediment across estuaries was not directly matched by contamination levels of biota, which in some cases were above the safety guidelines for consumption. It was clear however, that trace element content was more directly linked with ecophysiological attributes of different taxa. Mud snail, as a deposit feeder, accumulated trace metals most likely from ingesting food from a wide range of sources (decomposed matter), while seagrass accumulated more trace metals than sea lettuce due to the presence of a root system increasing metal bioaccumulation from sediments. Spatial data indicate that most metal sources were associated with freshwater input from streams, rivers and man-made structures such as storm-water drains and waste water ponds. The elemental composition of each pollution source was distinct and probably dictated by dominant land use in the catchment. Pb, Cu and Ni were more concentrated in urban areas and are typical contaminants of industrial waste. Cd and Zn had higher concentrations in the rural area, probably associated with intensive use of fertiliser in these agricultural catchments (Lambert, *et al.*, 2007). We demonstrated that trace elements can vary to a high degree across small spatial

ranges, which makes it difficult to predict an overall ecological impact/health status for a particular estuary. By combining results from different species, we demonstrate that additional ecological insight on pollutant characteristics can be deduced. However, accumulation rates of metals can be species specific and can be a function of size/age. Thus, caution is needed when interpreting results in order to avoid underestimation of the degree of impact that metal contamination may have on an ecosystem. While metal toxicity has been reported extensively in the literature, we found that background information was limited for *in situ* (field) scenarios and for species endemic to New Zealand. This research has provided an urban and rural baseline by which direct comparison with other estuaries around New Zealand is now possible.

3 Chapter Three

An assessment of trace element bioavailability along a terrestrial-estuarine-ocean transect using diffusive gradients in thin films

3.1 Introduction

It is necessary to monitor pollutants such as toxic trace metals (e.g. Cd and Pb) to satisfy the requirements of national guidelines, as such metals can pose a threat to human and ecosystem health. Estuaries are challenging environments in which to study trace element dynamics due to the influence of saline gradients, continuous tidal forcing together with changes in geochemical variables like potential of hydrogen (pH) and redox potential (Eh) between the sediments and water column. The potential redox is the characteristic of the chemical species to undergo an oxidation-reduction reaction. This study employs diffusive gradients in thin films (DGT) to develop an understanding of the extent of toxic, and non-toxic trace element availability across a terrestrial-estuarine-ocean transect.

The bioavailability of nutrients and toxic elements in estuaries is of direct relevance for water management because of their potential to influence trophic structure. These impacts may arise by stimulating excessive primary productivity (e.g. N and P pollution), and by affecting ecological integrity due to toxic effects (e.g. Cd, Pb). In the marine environment, trace element contaminants have traditionally been monitored in sediments and biota (Malea & Kevrekidis, 2013; Marsden, *et al.*, 2014; Tornero *et al.*, 2014). Bivalve molluscs represent one of the most frequently used spatial and temporal trend indicators of contaminants in aquatic environments (Norkko *et al.*, 2006). Sediments can act as a source or sink

for contaminants and often reflect localised sources of contamination from industrial and urban waste sources.

The solubility, mobility and bioavailability of sediment-bound metals can be increased by four major variables in terrestrial and aquatic environments:

- lowering of pH, for example, due to higher rates of organic matter mineralisation in aerobic surface waters (Kashem & Singh, 2001);
- increasing salt concentrations by the effect of competition on adsorption sites on solid surfaces and by the formation of soluble chloro-complexes with some trace metals (e.g. Cd) (Paalman *et al.*, 1994);
- increasing occurrence of natural and synthetic complexing agents (Wang *et al.*, 2015a);
- changing redox conditions, for example, after land deposition of polluted anoxic dredged materials (Cappuyns & Swennen, 2005).

Trace element concentration data from sediments are rarely representative of pollutant concentrations in the overlaying water column or in biota at higher trophic levels of food chains (Vink, 2009). Water concentrations often yield a clearer picture of the spatial distribution of contamination with relevance for the higher levels of the food chains. However, seawater samples pose an analytical challenge because of the extremely low concentrations of dissolved contaminants and high ionic strength of the sample matrix, which pose significant challenges to trace element quantification by standard methods such as inductively coupled plasma mass spectrometry (ICP-MS). This study circumvents the issues associated with direct trace element determination in salt water samples by using

diffusive gradients in thin films (DGT) (Zhang & Davison, 1995; Davison & Zhang, 2012).

Since toxicity and mobility of trace metals are related to the metals' speciation, robust methods for trace metal speciation analysis are of great interest (Allen & Hansen, 1996; Renner, 1997). In the last 20 years, toxicity assessment of dissolved metals has been explored using the DGT technique. This method has been extensively validated and has been applied to waters, sediments and soils (Davison & Zhang, 1994). However, the use of DGT in estuarine water is still limited compared to other techniques such as direct water sampling that have been used in more places and have been used to develop longer time series. DGT samplers have various advantages for environmental applications because they provide *in situ* measurements, are physically robust, and can be applied in a wide range of aquatic environments to detect a large range of elements simultaneously (Schintu *et al.*, 2010; Wu *et al.*, 2011). DGT measurements integrate time-weighted average concentrations during the deployment time which increases the sensitivity of analyses and provides information that temporally discrete sampling cannot. The diffusion process detects free ions or weakly bound complexes able to migrate to the resin layer (Zhang & Davison, 2000). This is comparable to migration across a cell membrane and so DGT-labile metal concentrations may represent the bioavailable metal fraction more closely than dissolved or total metal (Zhang & Davison, 2000). The evaluation of trace metal bioavailability has been compared using DGT and a chemical equilibrium model (WHAM 7.0) to examine the extent to which modelled speciation reflects field measurements (Han *et al.*, 2013). Han *et al.*, (2013) found that both methods predicted bioavailable concentrations for Ni, Zn and Cu but not for Pb. Trace metal bioavailability has also been assessed using macroalgae in conjunction with DGT (Schintu, *et al.*,

2010). Algae and DGT may give similar results of the spatial extent of contaminants, demonstrating multiple advantages of the use of bioindicators in conjunction with DGT in environmental monitoring (Raimundo *et al.*, 2011). Another advantage of using DGT with different layer thicknesses is the control of the extent of complex dissociation kinetics (Yapici *et al.*, 2008; Wu, *et al.*, 2011). The kinetics of dissociation of the metal complexes present are of fundamental importance in the study of the fate of trace elements in ecosystems. For small diffusion layer thicknesses (e.g. 0.4 mm), it is assumed that only free ions will be accumulated in the resin and the contribution of metals complexed by organic ligands is close to zero, as there is insufficient time for the complex to dissociate (Zhang & Davison, 2000). With increasing thickness, there is more time for complexes to dissociate, resulting to an increase in the concentration measured by DGT (Zhang & Davison, 2000).

In this research, trace elements including aluminium (Al), phosphorus (P), arsenic (As), chromium (Cr), manganese (Mn), barium (Ba), cadmium (Cd), lead (Pb) and uranium (U) in total, dissolved ($< 0.45 \mu\text{m}$) and DGT-labile fractions of fresh, estuarine, and oceanic water were determined.

The aim of this research was to monitor trace element composition of the estuarine and oceanic environment; to determine the magnitude of importance of natural vs anthropogenic sources of trace elements; as well as to gain an insight into trace element speciation and bioavailability in an urban estuary. The biological indicators selected for this study were seagrass (*Zostera marina*), sea lettuce (*Ulva lactuca*) and cockle (*Austrovenus stutchburyi*) as these species represented the most abundant and ecologically important taxa in the estuaries. Trace element concentrations in these species are also reported.

We hypothesise that trace metals in estuarine water would be higher than oceanic water due to the presence of freshwater inputs. The second hypothesis examines the concept that trace metals recorded in water samples from any particular area, would be higher than those recorded by DGT; as DGT represents an averaged time integrated measurement to assess the rate of bioaccumulation to ambient metal levels.

3.2 Method

3.2.1 Field measurements

Sampling was conducted within and outside the southern Tauranga Harbour, Bay of Plenty, New Zealand. The harbour is highly modified and is characterised by a high degree of land reclamation for industry and road networks. Land use in the surrounding catchment is a mixture of urban and agricultural, with 49% pasture, 9% orchards and crops and 8% exotic forest (Shaw, *et al.*, 2010). Six locations were sampled during the summer (January 2014) which captured distinct zones, likely to be affected by contrasting contamination sources (Fig. 3.1). The “Stream” site is located at the outlet of the Kopurererua stream in the Waikareao estuary and is affected by drainage from large industrial and urban areas. The “Bureta” site is located in the outer part of the Waikareao estuary and is potentially affected by a mix of urban and rural pollution sources and most likely by the marina and petrochemical manufacturing based industries located around the port area. The “Urban Bridge” site is located near the port and is representative of water influenced by large urban catchments. The “Pilot Bay” site is located near the port on the other side of the Bridge Urban site. The “Matakana” site is located further away from human activities, and is predominately affected by the surrounding rural environment as was shown in a previous hydrodynamic study (Tay, *et al.*, 2012). The “A beacon” site is located outside the Tauranga

Harbour and is therefore considered as a control site with minor human impacts. The Stream and Bureta sites were sampled again in the springtime (September 2014).

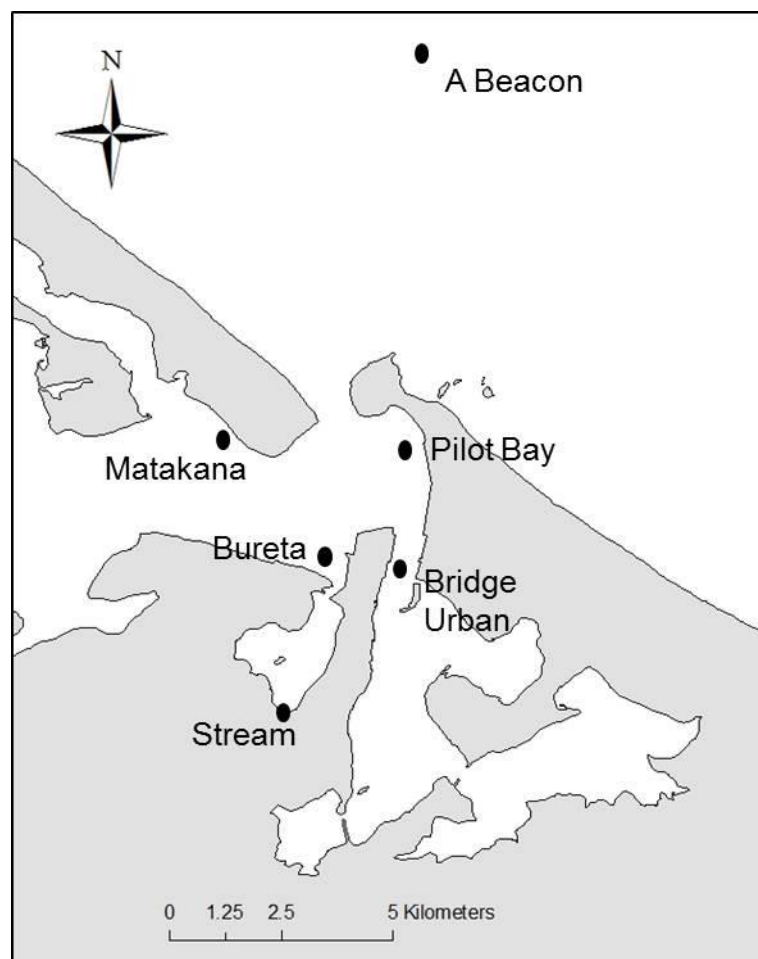


Fig. 3.1. Sample locations in the southern part of Tauranga Harbour.

3.2.2 Water Analysis

Water samples were collected 8 times in summer at six sites and 6 times in spring at Stream and Bureta for two tidal cycles (high tide and low tide) during the DGT deployment period. In addition, temperature, pH, Eh, salinity and conductivity were measured with a calibrated water sonde in the field (YSI Model 85, YSI incorporated) and calibrated pH meter (pHTestr 10, Eutech Instruments). Samples were syringe filtered ($<0.45\ \mu\text{m}$) and a 0.4 ml aliquot of total and filtered water was diluted with deionised water by a factor of 23 and acidified with 0.2 mL trace element grade HNO_3 . Samples were analysed on a Perkin Elmer

quadrupole ICP-MS calibrated using NIST-traceable standards (Inorganic Ventures, University of Waikato, Hamilton).

3.2.3 DGT-labile Trace Metals (*in situ* passive sampling)

Commercially available DGT units (supplied by DGT Research, Lancaster, UK) were deployed for 13 days in summer 2014 and 17 days in spring 2014. Each DGT device consists of a 25 mm diameter plastic backing cylinder and a front cap with a window of 20 mm in diameter. A layer of resin gel is placed on the base with the side containing the resin facing upward. A layer of diffusive gel is placed directly on the top of it (Fig. 3.2). One thickness (0.8 mm) in summer and three different thicknesses (0.4, 1.2 and 1.8 mm) of diffusive gel were used in spring. Trace elements were accumulated in the basal resin layer containing either Chelex-100 resin (cations), or a ferrihydrite resin gel layer (anions). Three replicate DGT units were placed at each location. On the recovery day, DGT units were rinsed with deionised water and placed into clean zip lock plastic bags ready for sample preparation in the laboratory.

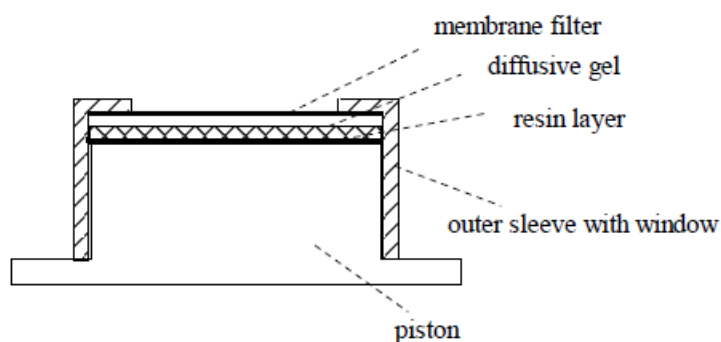


Fig. 3.2. Schematic representation of a section through the DGT device (Zhang et al, 1998)

In the laboratory, housings of the DGT units were removed and resin layers were carefully removed using plastic tweezers. Each resin was placed in a tube with 5 ml of 1M of HNO₃. The solutions were kept for 24 hours to allow

total dissolution. The solutions were then analysed using ICP-MS. The DGT-labile concentration of trace elements was calculated using the following formula:

$$C_{DGT} = \frac{M\Delta g}{DA t}$$

Where C_{DGT} is the measured DGT labile trace metal concentration; A is the exposed area of the DGT probe, Δg is the thickness of hydrogel; D is the diffusion coefficient which is water temperature dependant; t is the deployment time; and M is the mass of labile metal captured at the binding phase. D values were obtained from Lancaster DGT research except for As and U which were obtained from other published sources (Panther, 2008; Duquène *et al.*, 2010). Barium which was estimated based on the diffusion of Ba coefficient in seawater (Yuan-Hui & Gregory, 1974). The mass accumulated by DGT was calculated as follows:

$$M = \frac{C_{el}(V_{gel} + V_{HNO3})}{f_e}$$

Where, C_{el} is the concentration of the metals in the elution solution, V_{gel} is volume of the resin gel; V_{HNO3} is the volume of HNO_3 added to the resin and f_e is the elution factor (0.8).

3.2.4 Surface sediment and biota

Tissues of mud snails and cockles and blades of sea lettuce and sea grass were dried in an oven at 60°C and ground with a mortar and pestle. Cockle shells were carefully cleaned and dried at 60°C. Biological species, bulk and fine sediments were analysed for trace elements by x-ray fluorescence (XRF), Spectro Xepos (DKSH New Zealand limited, Mairangi Bay).

3.3 Results and Discussion

3.3.1 Trace elements in water along a terrestrial-estuarine-oceanic gradient

Results summarising the overall water chemistry of each site (temperature, pH, Eh, dissolved oxygen and conductivity) are presented in Table 3.1. Over the 13 days deployment time for the summer period, the average temperature of surface water ranged from 17.9°C at the A beacon to 19.7°C at Bureta. The site with the greatest variability was the Stream with values ranging from a maximum of 22.2°C to a minimum of 16.7°C with a standard deviation of 1.9°C. Such variation has only a small effect on the value of the diffusion coefficient used in Equation (1) to calculate the solution concentration of elements from DGT. The lowest salinity was found at the Stream (~10 psu) consistent with dilution by freshwater. Bureta had a lower salinity (~28 psu) than the Urban Bridge, Pilot Bay, Matakana and the A Beacon (~30-32 psu). Similar fluctuations in conductivity were measured at Bridge Urban, Pilot Bay, Matakana and A Beacon (~48 mS). A smaller decrease was recorded for Bureta (~44 mS) and the lowest value was recorded at the Stream (14.8 mS). There was therefore a clear salinity gradient between the Stream and other sites explained by tidal intrusion. However, the decrease in conductivity and salinity was not linked with freshwater intrusion at Bureta, as this site was further away from any freshwater influence originating from rivers and streams. The pH of all sites was similar (average pH = 8.1) except at the Stream. The Stream was characterised by a slightly higher pH (average pH = 8.3) and a larger standard deviation. The averaged redox potential was the highest at Matakana (163 mV) and the lowest at Bureta (96 mV). Higher standard deviations were typical for Matakana with values as high as 335 mV and as low as 38 mV. However, it should be noted that redox potential has to be interpreted

qualitatively due to inaccuracies in measurement. In addition to the major redox sensitive elements Mn and Fe, some trace elements that also undergo redox transformations behave very differently with regard to reactivity, mobility and toxicity depending on their redox state (Hamilton-Taylor & Davison, 1995). The toxic effects of redox-sensitive trace elements, which include As and Cr, will vary depending on the oxidation state of the species in question. Four redox conditions can be differentiated based on Eh values: oxidising (400 ~ 550 mV), weakly oxidising (200 ~ 400 mV), weakly reducing (0 ~ 200 mV) and reducing (0 ~ -200 mV) (Vershinn & Rozanov, 1982). On average, values of Eh were consistent with weakly reducing conditions. However, analysis of discrete samples demonstrated the high variability of the estuarine environment with water present in the weak oxidation zone from time to time. These changes in redox potential only partly reflect changes in the solubility of dissolved oxygen caused by salinity variations since dissolved oxygen varied inversely with salinity between the freshest (Stream site (6.6 ± 1.3 mg/L)) and most saline sites (A Beacon (7.5 ± 0.5 mg/L)).

Table 3.1: Environmental water quality parameters measured in summer 2014 in the identified sampling points (averages \pm standard deviation)

| Location | Temperature (°C) | Conductivity (mS) | pH | Eh (mV) | DO (mg/L) | Salinity (psu) |
|--------------|---------------------|----------------------|---------------|---------------|---------------|-------------------|
| Stream | 18.5 ± 1.9 | 14.8 ± 17.5 | 8.3 ± 0.3 | 105 ± 56 | 6.6 ± 1.3 | 10 ± 10.5 |
| Bureta | 19.7 ± 1.3 | 43.9 ± 4.7 | 8.1 ± 0.1 | 96 ± 55 | 6.9 ± 0.6 | 28 ± 3 |
| Bridge Urban | 19.2 ± 1 | 47.0 ± 1 | 8.0 ± 0.1 | 130 ± 63 | 6.9 ± 0.4 | 30 ± 0.8 |
| Pilot Bay | 18.0 ± 0.6 | 48.4 ± 0.6 | 8.1 ± 0.1 | 138 ± 88 | 7.1 ± 0.4 | 32 ± 0.5 |
| Matakana | 18.9 ± 1.2 | 48.2 ± 0.6 | 8.1 ± 0.1 | 163 ± 128 | 7.3 ± 0.9 | 31 ± 0.4 |
| A Beacon | 17.9 ± 0.5 | 48.8 ± 0.1 | 8.1 ± 0.1 | 106 ± 81 | 7.5 ± 0.5 | 32 ± 0.1 |

Trace element concentrations displayed the greatest differences between locations in the DGT-labile fractions, rather than total and dissolved fractions (Fig 3.3, 3.4). High DGT labile concentrations were found for Al, Mn, P and U (only at the Stream site), indicating that these elements were in the form of inorganic

complexes, or labile organic complexes (Table 3.2). In contrast, Cd, Cu, Ni, Pb, Ba and U (excluding the Stream site) were not strongly accumulated by DGT (Table 3.2).

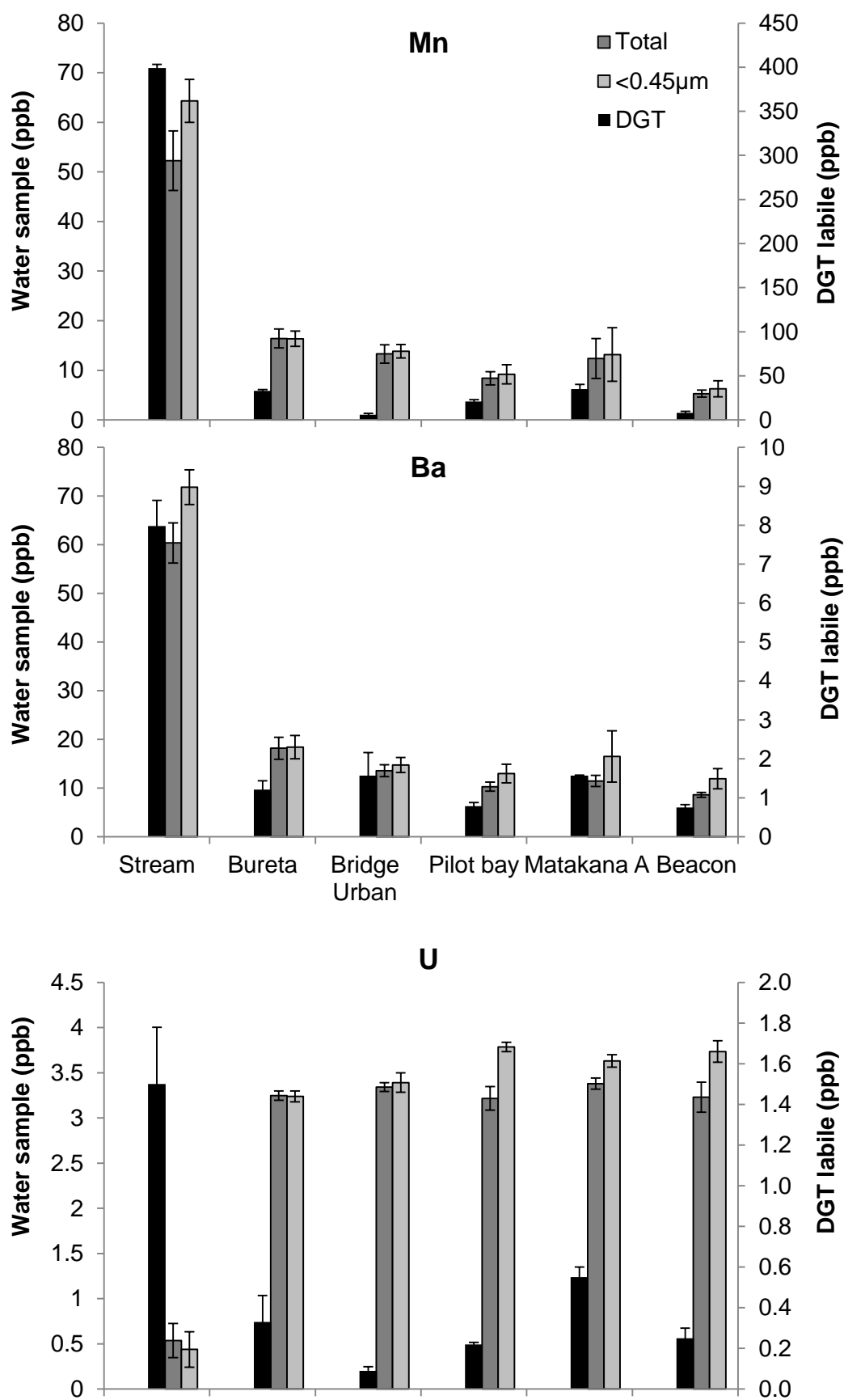
Table 3.2. DGT labile fraction of trace elements per site in summer 2014 ($C_{DGT}/C_{dissolved}$)

| Site Location | Al | Cr | Cd | Cu | Mn | Ni | Pb | As | P | Ba | U |
|---------------|------|------|------|------|------|------|------|------|------|------|------|
| Stream | 3.22 | 0.09 | 0.01 | 0.34 | 6.20 | 0.10 | 0.13 | 0.61 | 0.30 | 0.11 | 3.42 |
| Bureta | 2.26 | 0.10 | 0.02 | 0.03 | 2.02 | 0.05 | 0.07 | 0.15 | 0.27 | 0.07 | 0.10 |
| Bridge Urban | 2.43 | 0.04 | 0.01 | 0.02 | 0.43 | 0.02 | 0.03 | 0.20 | 0.43 | 0.11 | 0.03 |
| Pilot bay | 0.41 | 0.06 | 0.02 | 0.04 | 2.29 | 0.09 | 0.12 | 0.17 | 0.52 | 0.06 | 0.06 |
| Matakana | 0.72 | 0.10 | 0.06 | 0.17 | 2.66 | 0.13 | 0.07 | 0.35 | 1.29 | 0.09 | 0.15 |
| A Beacon | 0.18 | 0.03 | 0.04 | 0.04 | 1.27 | 0.11 | 0.10 | 0.19 | 1.09 | 0.06 | 0.07 |

Labile concentrations measured by DGT were higher at the Stream for Ba, Mn, U and Pb (Fig. 3.3). Most of the Ba, Mn, U and Pb in the water was present in the dissolved fraction whereas ~50% of the Al was present in particulate form, possibly as coarse colloidal or particulate aluminium oxides (Benoit *et al.*, 1994). Ba and Mn concentrations showed close similarities in their variation between sites, with higher concentrations measured at the Stream in DGT-labile, total and dissolved fractions (Fig. 3.3). At the Stream, DGT-labile concentrations of Ba were ~8 fold smaller than the dissolved concentration, but DGT-labile Mn was ~8 fold higher than its dissolved concentration. Mn and Ba concentrations in water and DGTs were similar for Bureta, Bridge Urban, Pilot Bay, Matakana and A Beacon with ~4 fold lower values compared to the Stream. Mn has similar chemical characteristics to Fe, partitioning between insoluble oxides and soluble dissolved ions as a function of electron activity and pH. It is possible that the conversion of solid oxides of Mn to reduced, dissolved Mn^{2+} in Stream sediments may also liberate other trace metals absorbed or bound to at their surfaces (Luther *et al.*, 2015). Most of the Mn was found in the DGT-labile fraction, which is

indicative of active reduction of Mn oxides, or a labile fraction of Mn associated with organic complexes. Mn and Ba were found only in small concentrations at the “Urban Bridge” (Fig. 3.3). Thus the large increase of Ba and Mn at the Stream is likely to reflect a terrigenous sedimentary input at this site. Aluminium concentrations in the DGT-labile fraction were higher at the Stream (580 ppb) and Urban Bridge (400 ppb) and lower at the A-Beacon site (34 ppb). Total and dissolved concentrations did not show any important variations at different locations. Al concentrations recorded in DGTs were 2 fold higher than water samples. Al concentrations appeared to be mainly related to terrestrial inputs, such as sedimentation events. Pb concentrations in DGT-labile fractions were highest at the Stream (1.06 ppb), being ~2.5 times greater than the control (A Beacon).

Dissolved water samples from the Stream had higher concentrations of Pb (7.2 ppb) with practically no variation being detected at the other locations (4 ppb), with the exception of Matakana (1.5 ppb). The variation in Pb with location was different from Aluminium. DGT-labile Pb was low at the Urban Bridge, which may indicate different sources for Pb and Al (sedimentary origin). Lead contamination at the Stream site was most likely to be due to point source discharge near the outlet of the stream as well as stormwater outlets as inferred previously (Chapter 2). U concentrations in DGTs and water samples showed opposite fluctuations between locations (Fig. 3.3). DGT-labile U was higher at the Stream (1.5 ppb) and Matakana (0.5 ppb) and lower at the Urban Bridge (0.09 ppb). Conversely, U in water samples was lowest at the Stream site (0.5 ppb) and higher at all other locations (~1.5 ppb). This is expected because U forms a stable carbonate complex in seawater and is therefore more abundant than in freshwater due to the higher alkalinity of the oceans (Pashalidis *et al.*, 1997).



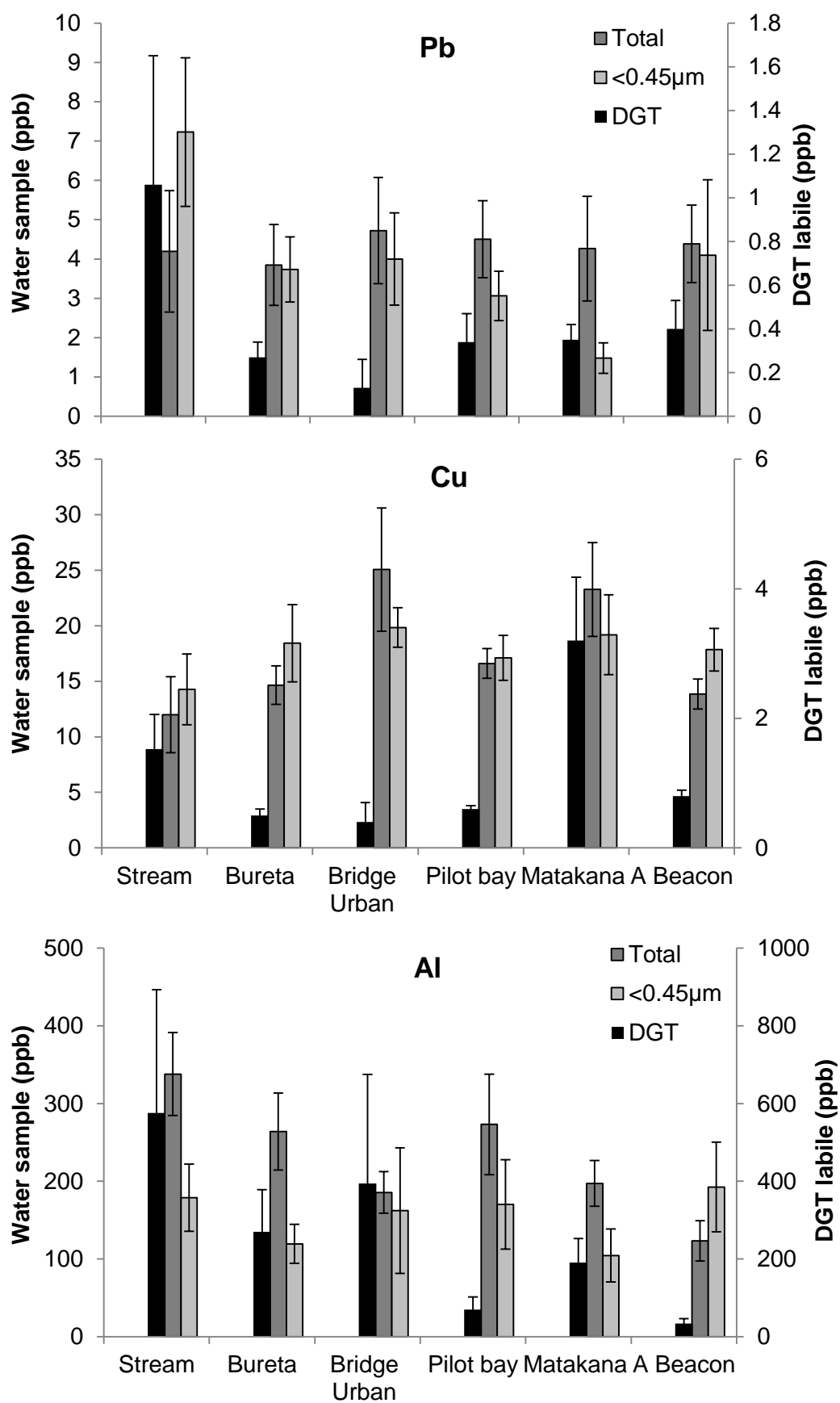


Fig. 3.3. Ba, Mn, U, Cu, Pb and Al (ppb) in DGT-labile, total and dissolved fractions from the Tauranga Harbour in summer 2014.

Cr and Cd in the DGT-labile fraction displayed similar fluctuations with higher concentrations at Bureta and Matakana (1 ppb for Cr and ~0.3 ppb for Cd) and lower concentrations at the A beacon site (0.3 ppb and 0.1 ppb respectively) (Fig. 3.5). Cr and Cd measured in water samples did not show any clear variation with most of the Cr and Cd present in the dissolved fraction with the exception of Cr at Pilot Bay. Particle-associated Cr ($Cr_{total} - Cr_{0.45}$) at Pilot Bay was 2 fold higher than the other sites, representing 50% of the Cr concentration in the bulk solution. This finding could be due to a local source of contamination near this area; potentially the use of CCA as a wood preservative and containing copper, chromium and arsenic. The general similarities between the distributions of Cd and Cr (Fig. 3.4A) could be linked to common origins associated with the agricultural sector, e.g. from intensive use of agrichemicals in the rural area around Matakana. Another potential source is fertiliser production and/or the treatment of wood timber at the port. As, P and Cu measured in DGT-labile fractions displayed similar fluctuations with higher concentrations recorded at Matakana (Fig. 3.5). This reinforces the prior interpretation made regarding Cr and Cd in terms of the agricultural impact on Tauranga Harbour as well as the release of chemicals during the manufacturing of fertiliser and/or the use of CCA wood preservative. DGT-labile Cu was higher at the Stream (1.5 ppb) compared to other sites (0.6 ppb), except for Pilot Bay. This indicates the possible terrestrial origin of Cu as well as the previous sources as mentioned above. However, it is important to note that neither Cu nor As are correlated with Cr, probably discounting CCA treated timber as an important source of these elements in the estuary (Fig 3.4A, D).

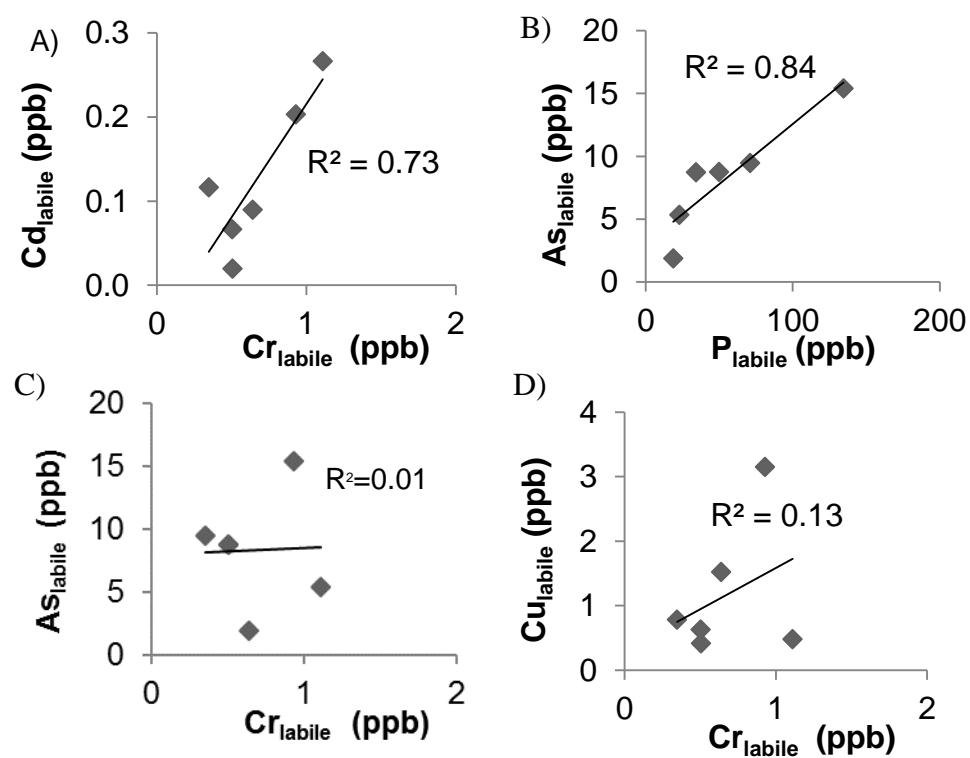
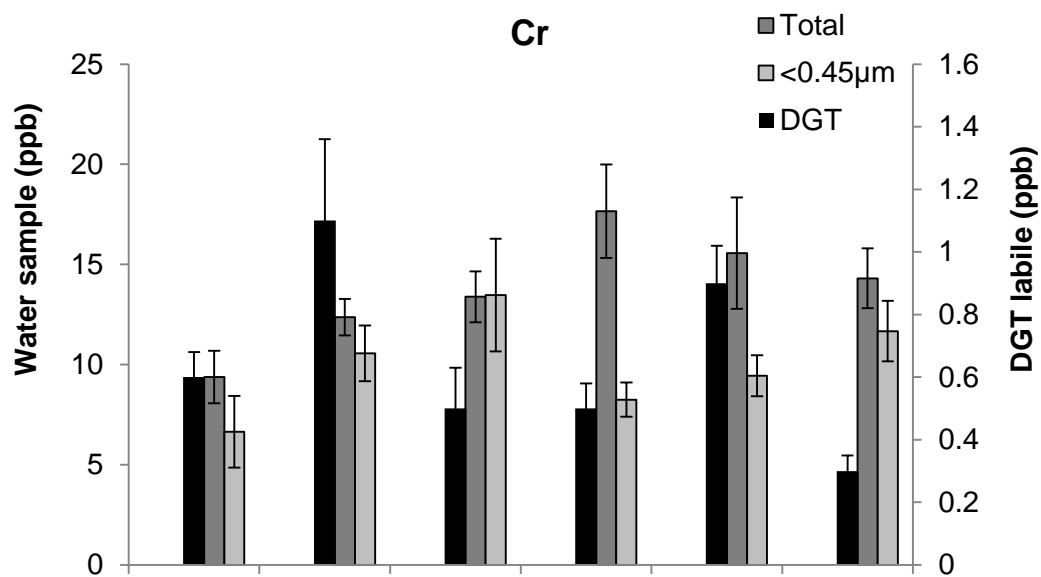


Fig. 3.4. DGT-labile concentrations of Cd vs Cr (A), As vs P (B), As vs Cr (C) and Cu vs Cr (D)



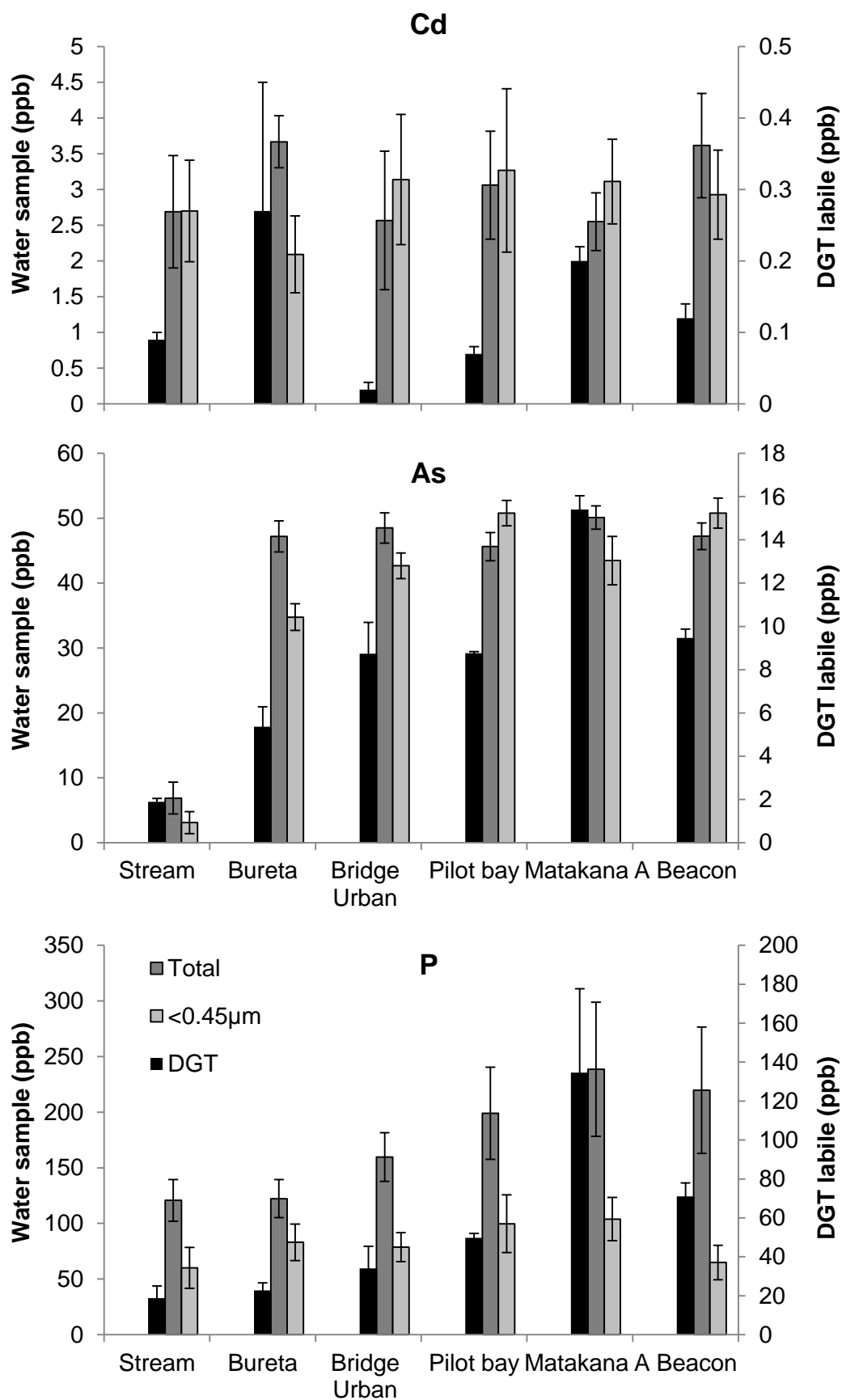


Fig. 3.5. Elemental concentrations (ppb) in DGT-labile, total and dissolved fractions from the Tauranga Harbour in summer 2014.

3.3.2 Trace element speciation in estuarine water and effects on benthic community

Results summarising the physical-chemical water proprieties in spring (temperature, pH, Eh, dissolved oxygen and conductivity) are presented in Table 3.3. Over the deployment time for the spring period, the average temperature of surface water was higher at Bureta (14.1°C) than the Stream (13.7°C) due to freshwater inputs from terrestrial run off at the Stream site. This was confirmed by examining water temperatures between low tide and high tide, since during high tides water temperatures were higher by ~1°C. Averaged salinity was higher at Bureta (25 psu) than Stream (12 psu). At the Stream, salinity was associated with terrestrial runoff with lower salinity measured during low tide (0.5 psu) than high tide (22.5 psu). Averaged conductivity followed salinity fluctuations with higher values found at Bureta (22.5 mS) than Stream (18.1 mS). Differences due to the tidal cycle showed small variability between high and low tide for Bureta (~4 mS) but high variability for the Stream (~34 mS). High variability at the Stream could be explained by lower conductivity during low tide (1 mS) compared to high tide (35.2 mS). Bureta had lower averaged pH (8) than the Stream (8.3) with smaller variation (~0.1). At the Stream, pH values displayed more differences with tidal cycle with higher pH recorded in low tide (8.8) than high tide (7.9), possibly due to the effluent water from local industries. Potential redox was higher at the Stream (161 mV) than Bureta (119 mV) with higher variation found at the Stream site (25 mV). At the Stream, fluctuations in the potential redox were associated with higher values during low tide (185 mV) than high tide (145 mV).

Table 3.3. Environmental water quality parameters measured in spring 2014 during low tide (LT) and high tide (HT) at the Stream and Bureta (mean values \pm Standard deviation).

| Location | Tide | Temperature °C | Conductivity (ms) | pH | Eh (mv) | DO (mg/L) | Salinity (psu) |
|----------|---------|-------------------|----------------------|---------------|--------------|---------------|-------------------|
| Stream | LT + HT | 13.7 \pm 1.1 | 18.1 \pm 19 | 8.3 \pm 0.5 | 161 \pm 25 | 8.7 \pm 2.1 | 12 \pm 12 |
| | HT | 14.2 \pm 1.3 | 35.2 \pm 4.3 | 7.9 \pm 0.2 | 145 \pm 7 | 8.6 \pm 0.8 | 22.5 \pm 3.5 |
| | LT | 13.1 \pm 0.3 | 1.0 \pm 1.2 | 8.8 \pm 0.1 | 185 \pm 21 | 8.8 \pm 3.2 | 0.5 \pm 0.7 |
| Bureta | LT + HT | 14.1 \pm 1.0 | 22.5 \pm 5.7 | 8.0 \pm 0.1 | 119 \pm 11 | 9.7 \pm 0.7 | 25 \pm 2.1 |
| | HT | 14.1 \pm 1.2 | 38.3 \pm 6.1 | 7.9 \pm 0.1 | 117 \pm 1 | 9.6 \pm 1.0 | 25.7 \pm 2.3 |
| | LT | 14.2 \pm 1.1 | 34.3 \pm 5.5 | 8.0 \pm 0.1 | 121 \pm 20 | 9.7 \pm 0.3 | 23.9 \pm 1.9 |

The following sections discuss the experimental data from deployments of multiple DGT samplers with varying diffusive gel thicknesses at the Stream and Bureta sites. This analysis allows the discrimination of three different sets of metal accumulation curves, characterised by their respective concentrations and fluxes as a function of diffusive boundary layer thickness (Δ_g) which equates to the thicknesses of diffuse gel layers (Fig. 3.6) (Wu, *et al.*, 2011):

- Non-sustained system: kinetic limited due to minimal resupply of trace elements by complexes following an initial accumulation of free ions. Characterised by a decrease in concentration and flux with hydrogel thickness;
- Partially sustained system: kinetic limitation is smaller. The resupply of trace elements from labile complexes occurs to some degree, as showed by a marginal increase in elemental concentration with hydrogel thickness;
- Fully sustained system: no kinetic effect due to a constant resupply of metals due to diffusion of free ions and labile complexes, resulting in an increased concentration and a constant flux with increased hydrogel thickness.

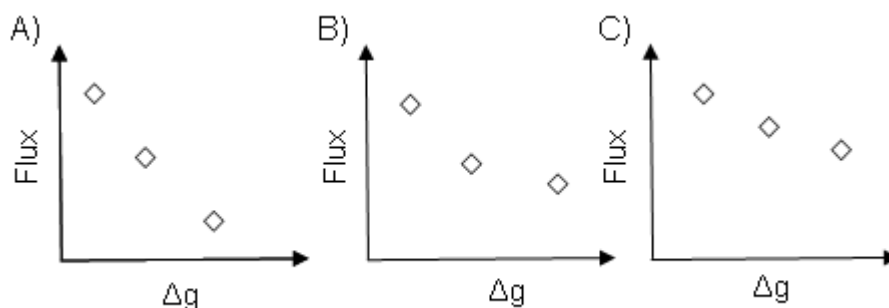


Fig. 3.6. Conceptual diagram describing the accumulation profiles of trace elements by DGT as a function of hydrogel thickness (Δg) for: non-sustained (A), partially sustained (B) and fully sustained (C) systems. These systems show a decreasing degree of kinetic limitation (from A to C) as labile trace element complexes contribute to increasing extents to the flux measured by DGT.

At the Stream site, Cr, Cd, Al, Ni, Cu, Fe, As, P, Zn and Pb were characterised by a partially sustained behaviour consistent with a limited degree of resupply, either from the solid phase or from dissolved ligands (Appendix I, Fig. 3.8). Variations in DGT flux and C_{DGT} for Fe, Al, Cr, Cd, Ni, Cu and Pb were similar between the different hydrogel thicknesses, but not As, P, and Zn. We may speculate that these elements were closely associated e.g. by adsorption to Fe and Al oxide surfaces and subsequently dissolution is the driving mechanism for covariation. Mn and Ba were characterised by fully sustained behaviour consistent with a constant degree of resupply of free ions and labile complexes (Appendix I, Fig. 3.8). At Bureta, Cd was characterised by a non-sustained system consistent with no resupply from complexes (Appendix I, Fig. 3.8). Mn, Pb, Ba, As and P were characterised by partially sustained behaviour consistent with a limited degree of resupply. Cr, Al, Ni, Cu, Fe and Zn were characterised by a fully sustained behaviour consistent with a constant degree of resupply of free ions and labile complexes. Comparisons of trace elements under different tidal cycles (Table 3.5) demonstrated that Al, Fe, Mn originated from the Stream and Bureta was the main source for Ba, P, Cr, Cu, Zn and As with negligible difference between totals and dissolved fractions.

Ni, Fe, Pb, Cu, Cr, Mn and Zn in biota were higher at the Stream which was consistent with DGT-labile fluctuations (Table 3.4A, B; Appendix I, Fig. 3.8, 3.9). Interestingly, DGT_{1.18} Cu and Cr concentrations were well correlated with Cu and Cr accumulation by sea lettuce and Al, Cu, Ba and Pb in DGT_{1.18} were well correlated with accumulation by seagrass (Appendix I, Fig. 3.10). However, Al in biota was higher at the Stream which contradicted DGT_{0.39, 1.96} results, however they were similar to DGT_{1.18}, water sample and surface sediment results (Table 3.4A, B; Appendix I, Fig. 3.9). As and P exhibited the same behaviour between biota and DGT with higher concentrations at Bureta except for seagrass which was found to have concentrations at the Stream for both elements (Table 3.4A, B, Appendix I, Fig. 3.8, 3.9). This could be due to recycling of past contaminants by the roots as mentioned in Chapter 2. It is well known that iron oxides exert important controls on the cycling of P and As in many aquatic systems, due to release of P and As following the dissolution of Fe oxides (Hossain *et al.*, 2008; Yang *et al.*, 2015a). Interestingly, DGT_{1.18} As concentrations were well correlated with As accumulation by cockle tissues (Appendix I, Fig 3.10). P, Ni, Zn and Mn in DGTs were not significantly correlated with biota probably due to internal metabolism as essential elements for life (Appendix I, Fig 3.10).

Table 3.4. Comparison of trace elements in water (A) and biota (B) between the Stream and Bureta. Shaded area represents the highest averaged concentration between sites. nd: not detectable, SS: surface sediment, SG: seagrass, SL: sea lettuce, C: cockle, W: water

| A | Stream | | | | | Bureta | | | | |
|----|--------------------|-------------------------|---------------------|---------------------|---------------------|--------------------|-------------------------|---------------------|---------------------|---------------------|
| | W _{total} | W _{<0.45µm} | DGT _{0.39} | DGT _{1.18} | DGT _{1.96} | W _{total} | W _{<0.45µm} | DGT _{0.39} | DGT _{1.18} | DGT _{1.96} |
| As | | | | | | | | | | |
| P | | | | | | | | | | |
| Ni | | | | | | | | | | |
| Fe | | | | | | | | | | |
| Al | | | | | | | | | | |
| Pb | | | | | | | | | | |
| Cu | | | | | | | | | | |
| Cr | | | | | | | | | | |
| Mn | | | | | | | | | | |
| Zn | | | | | | | | | | |
| Ba | | | | | | | | | | |
| Cd | | | | | | | | | | |

| B | Stream | | | | | | Bureta | | | | | |
|----|---------------------|--------------------------|----|----|---------------------|--------------------|---------------------|--------------------------|----|----|---------------------|--------------------|
| | SS _{total} | SS _{<0.64µm} | SG | SL | C _{tissue} | C _{shell} | SS _{total} | SS _{<0.64µm} | SG | SL | C _{tissue} | C _{shell} |
| As | | | | | | nd | | | | | | nd |
| P | | | | | | | | | | | | |
| Ni | | | | | | | | | | | | |
| Fe | | | | | | | | | | | | |
| Al | | | | | | | | | | | | |
| Pb | | | | | | | | | | | | |
| Cu | nd | | | | | | nd | | | | | |
| Cr | | | nd | | | | | | nd | | | |
| Mn | | | | | | | | | | | | |
| Zn | | | | | | | | | | | | |
| Ba | | | | | | | | | | | | |
| Cd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

PCA analysis including trace elements in particulate and dissolved forms under different tidal regimes explained 65% and 73% of total variation respectively, and permitted an examination of the degree of localisation of contaminant sources (Fig. 3.7.A, B). At the Stream during the low tide, trace elements in particulate and dissolved forms were clearly separated. PCA analysis demonstrated that Ba and Mn were the elements most related to terrestrial inputs, with a possible terrestrial effect for Pb, Fe, and Al. Whether the higher concentration of Ba in DGTs from the Stream simply reflects greater Ba availability at this site, or conversely reflects greater competitive exclusion of Ba at Bureta, remains unknown. Cr, As and Ni in particulate and dissolved forms appeared to be mostly associated with the intrusion of oceanic or rural water in the estuary. However, for the Stream, PCA analysis of trace elements in the dissolved

fraction showed a different pattern during high tide than the particulate fraction. A strong association was evident between Cu, P, Pb and Al which indicates that these elements were not related to terrestrial input or the intrusion of oceanic/rural water. This last group of trace elements (Cu, P, Pb and Al) most likely originated from other sources probably present in the proximity of our sampling site (Stream), as for example the stormwater outlet responsible for Pb inputs (identified in Chapter 2). Zn was closely associated to Cd and most likely related to agricultural or industrial waste (Copius Peereboom-Stegeman & Copius Peereboom, 1989; Schipper, *et al.*, 2011).

PCA analysis including DGT-labile forms explained 79% of total variation (Fig. 3.7C). At the Stream, DGT_{1.96} displayed smaller affinities in the accumulation of trace elements than DGT_{1.18}, characteristic of a partially sustained system with a limited degree of resupply, either from dissolved ligands or from the solid phase. At Bureta, an increasing supply of trace elements was observed with thicknesses, most likely associated with a constant resupply from free ions or labile complexes. However, this accumulation pattern was less evident for Cd. Most of the trace elements were correlated with Fe, excluding Cd, indicating that their presence was dependant of Fe-containing oxide minerals in the Waikareao estuary. Al (like Fe) also forms a hydroxide at higher pH values typical of these systems, and therefore is rapidly removed from the water column by destabilisation of colloidal suspensions (Upadhyay, 2008; Neubauer *et al.*, 2013). Furthermore, P and As were closely associated to Al and their mobility could be associated to Al adsorption sites and hydroxide dissolution (Rydin *et al.*, 2000).

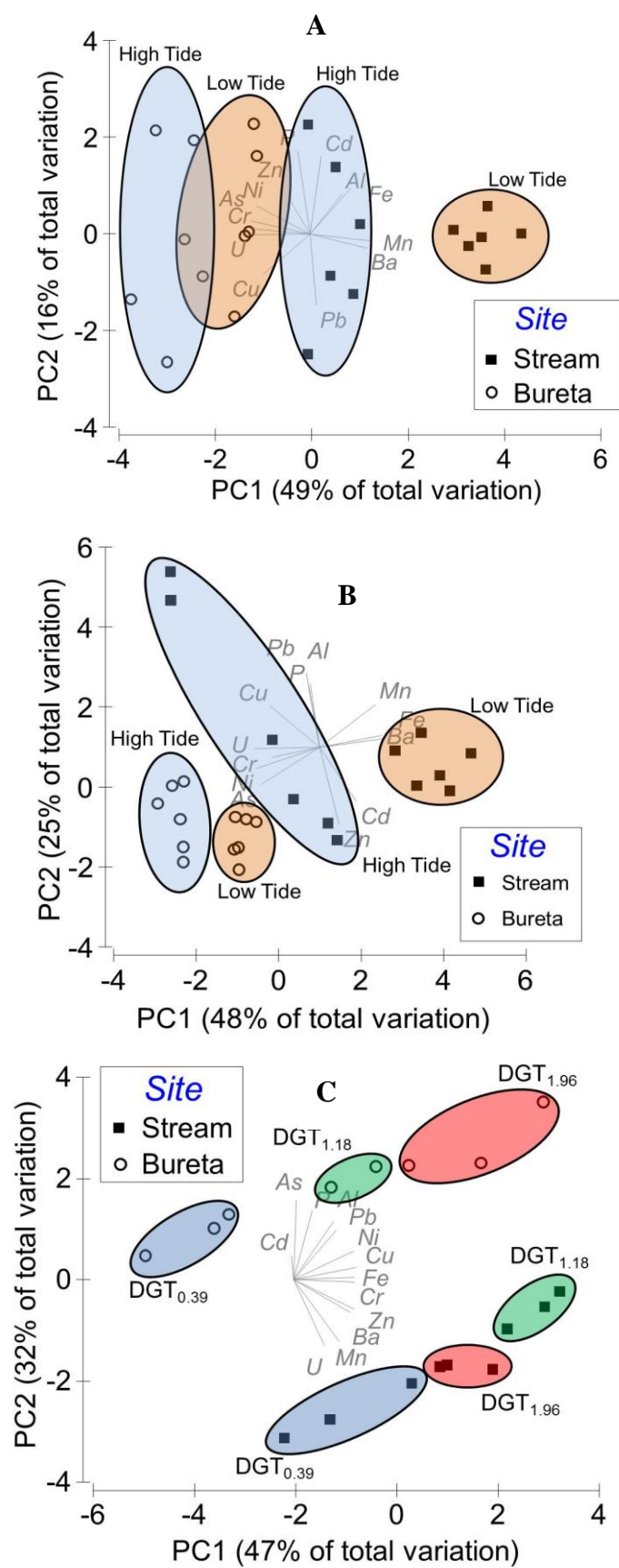


Fig. 3.7. Principal component analysis of trace elements in bulk water sample (A), in dissolved fraction (B) and DGT-labile (C)

Table 3.5. Total and dissolved elemental concentrations in water sample during high tide (HT) and low tide (LT) at the Stream and Bureta

| Location | Tide | Al | | P | | Cr | | Fe | | Mn | |
|----------|------|--------------|-------------------|--------------|-------------------|--------------|-------------------|--------------|-------------------|--------------|-------------------|
| | | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> |
| Stream | HT | 309 ± 22 | 338 ± 143 | 70 ± 15 | 132 ± 32 | 5.7 ± 0.5 | 6.7 ± 0.6 | 284 ± 39 | 26 ± 18 | 71 ± 4 | 70 ± 16 |
| | LT | 463 ± 73 | 141 ± 29 | 57 ± 7 | 63 ± 10 | 2.0 ± 0.3 | 2.9 ± 0.7 | 846 ± 132 | 290 ± 15 | 169 ± 23 | 164 ± 22 |
| Bureta | HT | 310 ± 74 | 141 ± 40 | 83 ± 16 | 70 ± 14 | 7.6 ± 0.4 | 7.6 ± 0.4 | 126 ± 83 | 0 | 8 ± 2 | 5 ± 0.37 |
| | LT | 342 ± 82 | 86 ± 9 | 90 ± 11 | 56 ± 9 | 6.5 ± 0.4 | 6.2 ± 0.4 | 121 ± 76 | 0 | 21 ± 3 | 21 ± 2.6 |
| | | Ni | | Cu | | Zn | | As | | | |
| | | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | | |
| Stream | HT | 6.5 ± 0.7 | 6.5 ± 0.5 | 17.5 ± 0.7 | 19.4 ± 2.2 | 57 ± 5 | 66 ± 11 | 21.1 ± 4.1 | 18.6 ± 2.9 | | |
| | LT | 2.5 ± 0.2 | 2.7 ± 0.3 | 14.9 ± 0.6 | 16.0 ± 1.4 | 44 ± 3 | 54 ± 5 | 1.4 ± 0.7 | 0.7 ± 0.4 | | |
| Bureta | HT | 19.9 ± 8.6 | 11.2 ± 0.9 | 32.3 ± 9.2 | 21.0 ± 0.8 | 141 ± 78 | 67 ± 10 | 67.0 ± 6.3 | 64.5 ± 9.7 | | |
| | LT | 9.6 ± 0.5 | 8.3 ± 0.5 | 19.4 ± 0.6 | 18.5 ± 0.6 | 50 ± 4 | 55 ± 5 | 44.6 ± 5.2 | 46.1 ± 6.7 | | |
| | | Cd | | Ba | | Pb | | U | | | |
| | | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | <i>Total</i> | <i><0.45μm</i> | | |
| Stream | HT | 0.1 ± 0.0 | 0.4 ± 0.2 | 66 ± 4 | 46 ± 8 | 2.7 ± 1.4 | 3.8 ± 1.5 | 1.2 ± 0.1 | 2.2 ± 0.7 | | |
| | LT | 0.4 ± 0.1 | 0.5 ± 0.1 | 73 ± 3 | 66 ± 3 | 2.0 ± 0.3 | 2.1 ± 0.5 | 0.1 ± 0.0 | 0.1 ± 0.0 | | |
| Bureta | HT | 0.2 ± 0.1 | 0.2 ± 0.0 | 15 ± 2 | 14 ± 1 | 3.6 ± 1.6 | 2.8 ± 1.4 | 3.1 ± 0.1 | 3.2 ± 0.1 | | |
| | LT | 0.5 ± 0.1 | 0.5 ± 0.1 | 24 ± 3 | 26 ± 2 | 1.7 ± 0.2 | 1.2 ± 0.4 | 2.6 ± 0.1 | 2.3 ± 0.1 | | |

3.4 Conclusion

In this study, the distribution of trace elements along a stream-estuary-oceanic gradient was investigated using discrete water samples and time-integrated DGT measurements. Generally, trace element concentrations in DGT-labile fractions showed similar fluctuations to water samples, with DGT measurements circumventing issues with low trace element concentrations in the estuarine water and ICP-MS matrix suppression by the high concentrations of dissolved solids in the water. Trace element concentrations in the DGT-labile fraction were higher within the estuary than the ocean, demonstrating the importance of anthropogenic and terrestrial inputs. In this study, an advantage of discrete water sampling was the ability to discriminate between high and low tides, permitting terrestrial inputs from the Stream (Mn, Ba, Al and Pb) to be separated from other diffuse/point sources of contamination.

Another aim was to characterise trace elements in terms of their degree of association with organic complexes or colloidal species, as indicated by the rate of accumulation by DGT samplers with different hydrogel thicknesses. The association of trace elements with organic complexes/colloids was inferred by calculating of concentration and flux of DGT-labile elements across different thicknesses. The system of classification adopted here (sustained > unsustained) permitted insights into the speciation of the various elements. This analysis showed a range of behaviour from probable simple hydrated and inorganic cations with no resupply from complex ligands (e.g. Cd at Bureta) to fully sustained conditions with a constant degree of resupply of free ions and labile complexes (e.g. Fe, Ni and Cu at Bureta). Trace elements in the DGT-labile fraction showed similar fluctuations to biota at the different locations, with stronger significance

found for sea grass and Al, Cu, Ba and Pb, cockle and As and sea lettuce with Cr and Cu. Safety guidelines for estuarine water (ANZECC, 2000) were exceeded for Zn, Cu and Ni in water sample in order of magnitude. A tidal deployment of DGT (6 hours duration) would be beneficial in the future to clearly determine the different sources of pollution affecting the Tauranga Harbour.

4 Chapter Four

Nutrient sources and limitations as investigated by stoichiometric and stable isotope ratios in Bay of Plenty Estuaries

4.1 Introduction

Estuaries, the physical interface between terrestrial and coastal environments, are defined as partly enclosed wet areas where water flow is reduced. As such they are prone to accumulation of nutrients and anthropogenically derived, frequently toxic chemicals, such as polychlorinated biphenyl (PCB), dichlorodiphenyltrichloroethane (DDT) and heavy metals (Glasby, *et al.*, 1990b). Estuaries are considered as highly productive ecosystems (Costanza, *et al.*, 1996) and are an important habitat for the ecology of many species including those that transition from freshwater to the sea and vice versa. Wetlands are particularly important as nursery grounds for native species (Sheaves, *et al.*, 2007; Davis, *et al.*, 2012) and are considered a natural ‘scrubber’ for the filtration of the water column, hence resulting in accumulation of many pollutants (Tack, *et al.*, 2007).

Nitrogen and phosphorus are essential nutrients for the growth of plants and animals, but are often present in short supply. In the heavily populated areas of the world coastlines, the anthropogenic inputs of P into the coastal ocean are 10-100 times greater than in pre-industrial times (Caraco, 1993). Both nitrogen and phosphorus are regularly applied through fertiliser to increase the yield of crops as well as being widely used for residential and commercial purposes. Additionally, nutrient inputs have been internationally linked with the disposal or

leakage of poorly treated wastewater (Lawrie *et al.*, 2010; Parker *et al.*, 2012). Natural sources including volcanic activities, geochemical properties of natural soils, as well as the coastal incursion of oceanic water during upwelling events, can be additional sources of nutrients (D'Croz & O'Dea, 2007; Cravo *et al.*, 2014). When nutrients are present in excess (eutrophication), they are considered as pollutants because of their numerous negative impacts on the environment. For example, higher concentrations of nitrogen and phosphorus can act as to enhance chlorophyll *a* production due to unbalanced extreme macro-algae growth that impacts the whole ecosystem (Menéndez *et al.*, 2002). The availability of nitrogen and phosphorus will influence the amount and composition of toxins produced by harmful algae blooms (Adolf *et al.*, 2009; Van de Waal *et al.*, 2009).

Blooms of macroalgae have been reported harmful on marine intertidal ecosystems due to possible changes in ecosystem structure and the reduction of biodiversity (Franz & Friedman, 2002). *Ulva lactuca* (here after, sea lettuce) can inhibit the growth of estuarine sediment surface microalgae (Nan *et al.*, 2008) and blanket parts of seagrass and shellfish beds (Kennish *et al.*, 2007). Rural areas in New Zealand are dominated by agricultural practices using large amounts of human-made nutrients on the land (Buckthought *et al.*, 2015). Blooms of sea lettuce in Tauranga Harbour are a visual characteristic of the estuary due to the increase of natural (upwelling) and anthropogenic nutrients within the ecosystem (Park, 1996; Huteau, 2009). Understanding the sources responsible for eutrophication in New Zealand estuaries is necessary to accurately determine future remediation steps to prevent any further environmental degradation.

Traditional methods in environmental monitoring often use sampling of physiochemical tracers, such as nutrients and salinity, or phytoplankton biomass.

These methods are laborious, time-consuming and costly, and often provide an inaccurate picture of the spatial dispersion of effluent. In well mixed water, the signals of these tracers are rapidly lost as the pollutant is diluted, and at best, they provide only an instantaneous view. Short pulses of pollution in areas that would not receive constant pollution are likely to be missed, although *en masse*, these circumstances may negatively impact the global ecosystem. The relatively recent use of stable isotope analyses in chemical ecology has permitted a novel approach to explain what classic monitoring methods fail to answer. Dual carbon and nitrogen stable isotope analysis is a useful tool for the tracking of nutrients and organic matters in aquatic systems and may indicate the dispersal of sewage nitrogen (Rogers, 1999; Savage & Elmgren, 2004; Savage, 2005; Hartland *et al.*, 2011; Rogers *et al.*, 2012).

The use of stable isotopes has been applied to environmental issues linked with eutrophication, as they permit the identification of nitrogen sources for a wide range of activities such as mining, wastewater and agriculture effluent discharge (Ronkanen & Kløve, 2007; Bruce & Jeffrey, 2010; Rogers, *et al.*, 2012). Any factor that influences the stable isotopic content of elemental sources will necessarily influence the stable isotopic composition of plants and invertebrates. Although commonly applied to study of sea lettuce in New Zealand (Barr, 2007), stable isotope analysis of sea grass and other benthic species, such as mud snails are limited in New Zealand. Knowing the isotopic ratio of a wider range of species will support further understanding of pollution effects as well as anticipate potential risks for predator species from higher trophic levels. Nitrogen isotopic ratio in tissues of macroalgae provide a useful indicator for the source of nitrate assimilated, and have been successfully used to assess the potential impacts of human activities, particularly sewage in aquatic environments (McClelland &

Valiela, 1998; Rogers, 1999; Umezawa *et al.*, 2002; Cohen & Fong, 2005; Rogers, *et al.*, 2012). Sewage effluent contains carbon and nitrogen of the sources that are isotopically distinct or higher compared to background isotopic signatures. Isotopic analysis can therefore be used for tracing terrestrial nutrient sources entering marine ecosystems. Higher $\delta^{15}\text{N}$ values due to sewage effluent have been correlated with higher concentration of nitrogen and phosphorus in sediment, water and macroalgae (Savage & Elmgren, 2004; Bruce & Jeffrey, 2010). Thus we predict in this study that higher $\delta^{15}\text{N}$ will be correlated with increases in total nitrogen and phosphorus in surface sediment. One of the advantages of the use of isotopic signatures of the tissue of bio-indicator species is that they provide time integrated measures of sewage exposure over the period of the organisms life time (Bode *et al.*, 2014; Žvab Rožič *et al.*, 2014). Effluent can be discharged into the harbour as a concentrated pulse over a short time period, and may well leave a trace within hard structures of marine organisms, but conventional water sampling will most likely fail to record such short term pollution events. However, the determination of the relationship between nitrogen concentration in surface water and the quantity of nitrogen introduced from a particular source is complicated due to the occurrence of multiple sources of nutrients in many areas, and the presence of overlapping point sources and diffuse sources. This complication can be resolved by comparing different isotopes from different indicator species to allow discrimination of nutrient sources (Rogers, 1999; Minet *et al.*, 2012).

The Tauranga Harbour catchment has an area of 200 km² with a variety of land uses including horticulture, agriculture, urban and industry. The Wairoa River constitutes the largest freshwater input into the harbour. As a response to one of the fastest growing cities in New Zealand, a wastewater treatment plant (WWTP) scheme was put in place to treat and discharge wastewater for Tauranga

city. The city was first reticulated for sewerage in 1915 with extension to the entire city in the 1960s. In 1979, a sludge conditioning pond was built in Te Maunga, on the edge of the Rangataua estuary. By 1983, waste was discharged into oxidation ponds in Te Maunga and the secondary treated sewage discharged to the open ocean through an offshore outfall. The WWTP has however been associated with a number of seepages from unlined ponds since it was built. These leaks discharge within ~20 m of the pond bund into Rangataua Estuary (TCC, 2004). Tauranga Harbour, therefore, constitutes a useful estuarine ecosystem to examine multiple and complex sources of pollution such as stormwater drains, wastewater from treatment plant and agriculture waste. Research undertaken by the Bay of Plenty Regional Council identified higher concentration of nitrogen in Kopurererua (Waikareao estuary) and Waimapu streams. Long-term measurement indicate that mean DIN concentrations were generally higher in winter while phosphate did not appear to follow a temporal pattern, and concentrations were generally five times lower than DIN concentrations. The nitrogen was mostly present in the form of TOx-N which may indicate that livestock agriculture is the major source (Scholes & McIntosh., 2009). The use of stable isotopes in Tauranga Harbour has been limited to a few studies mainly using sea lettuce as a species indicator. Barr (2007) has sampled sea lettuce in two urban sites in Tauranga Harbour for the elaboration of a New Zealand isotopic guideline of anthropogenic inputs (Barr *et al.*, 2013). Huteau (2009) sampled sea lettuce in 33 sites within Tauranga Harbour as well as offshore islands (Karewa and Tuhua) to track sources of nitrogen potentially responsible of sea lettuce blooms. He suggested an influence from deep water upwelling at Tuhua, but higher levels of $\delta^{15}\text{N}$ in the Harbour suggested an anthropogenic origin of nitrogen responsible for *Ulva* blooms (Fig. 4.1). Hailes (2006) measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in 13

different species at Tuapiro and Otumoetai in the Tauranga Harbour to determine the contribution of seagrass as a food source and found that seagrass plays a role in the production of benthic invertebrate communities. Seagrass was an exclusive food source for gastropods such as the mudflat topshell (*Diloma subrostrata*) and the horn shell (*Zeacumantus lutulentus*).

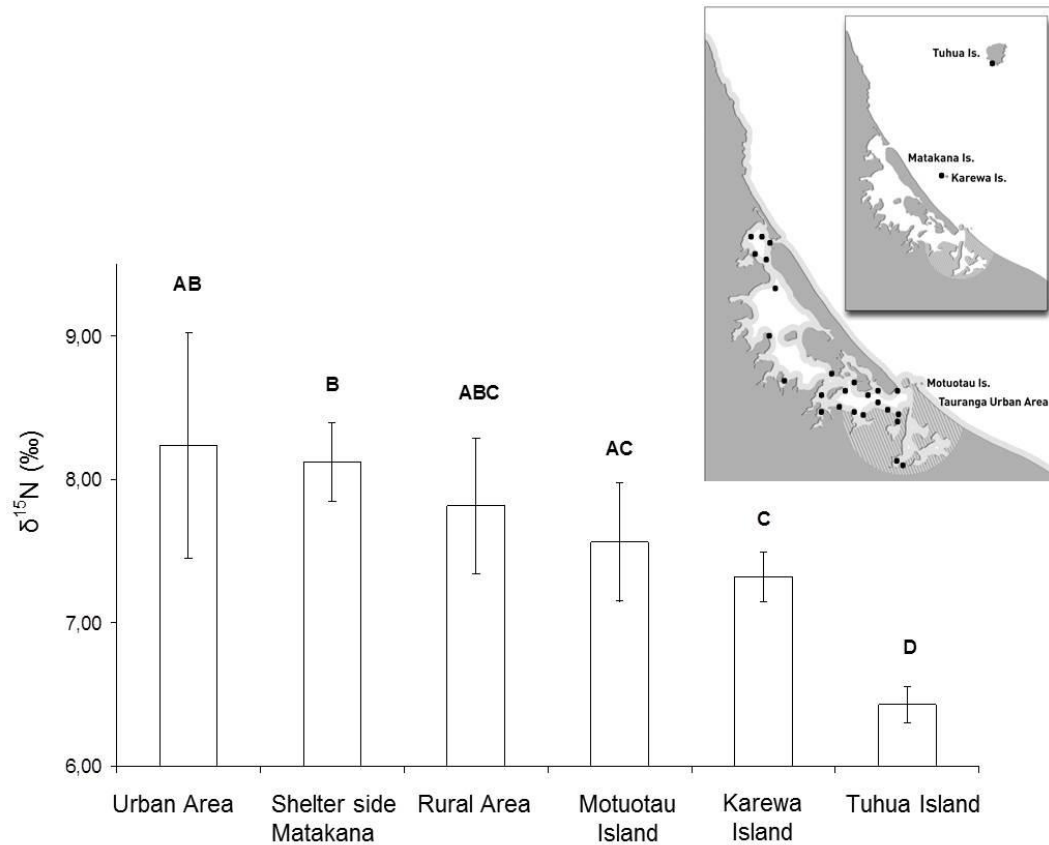


Fig. 4.1. $\delta^{15}\text{N}$ values (with 95% confidence intervals) found in sea lettuce from different parts of Tauranga Harbour and the open coast. Letters indicate significant differences between sample groups (Huteau, 2009).

It is widely accepted that in coastal waters, it is the availability of dissolved inorganic nitrogen that is most likely to constrain primary production (Ryther & Dunstan, 1971; Howarth & Marino, 2006). However, phosphorus (P) has been found to be the limiting nutrient in other areas such as the Yellow Sea, China (Zhang *et al.*, 2015) and some parts of the Baltic Sea (Andersson *et al.*, 1996). A switch from spring P to summer N limitation has also been demonstrated in some locations (Fisher, 1992; Conley, 1999). The determination of the limiting

nutrient is principally based on the study of mass balance between elements such as C, N, P and Si (Redfield ratio) in the water column (Neill, 2005; Li *et al.*, 2013; Zhang, *et al.*, 2015) and in some cases, compared to phytoplankton biomass (Wu & Chou, 2003; Domingues *et al.*, 2011). However, in Tauranga Harbour, there is no information on which nutrients have the most effect on the primary production. Here we report the first study for the Tauranga Harbour looking at stoichiometric ratio in mangrove, seagrass and sea lettuce in summer and winter. In this study, we choose to directly analyse tissues of sea lettuce and seagrass to understand their nutrients requirements and identify potentially limiting nutrients, which has been shown to be often species specific (Fong *et al.*, 2003), rather than measuring the ambient concentration in the water column. It allows us to examine potential competition in nutrient availability between the fast-growing opportunistic primary producers, i.e., sea lettuce and seagrass.

Ecological stoichiometry can be used to analyse factors affecting energy and material flows at the autotroph-primary and secondary interface in estuarine ecosystems. In this paper, we document the spatial variability of C, N, and P concentrations and stable isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in sediment and organisms. The first aim is to examine the food web structure by stable isotope analysis to understand prey-predator relations and possible pathways of contaminant accumulation related to diets (biomagnification) rather than location. Since isotope fractionation can be highly variable between different species (McCutchan *et al.*, 2003), we separately analysed the gut and muscle tissues of invertebrates to discriminate between freshly ingested food without any trophic fractionation (gut) and the isotopic ratio after trophic shift of the species (muscle). A second aim is to evaluate whether Tauranga Harbour is eutrophic and to elaborate on the use of stable isotope analyses to localise sources of nitrogen and phosphorus between

different stressed estuaries, with emphasis on the detection of wastewater seepage. The comparison of C:N:P ratio in autotrophs (seagrass, sea lettuce and mangrove) will permit assessment of whether nitrogen or phosphorus could limit the primary production in the Tauranga Harbour during the highly productive summer period.

We hypothesise that the isotopic ratio recorded in biota would be species specific due to differences in diet and origins of nutrients, with higher nitrogen ratio for species present in higher trophic level. However, the carbon and nitrogen ratio will fluctuate within the same species due to the presence and effect of different contaminant sources. Species affected by treated waste water will have higher carbon and nitrogen isotopic ratio. We hypothesise that higher nitrogen ratio in sediment would be correlated with higher concentration of total nitrogen.

4.2 Method

4.2.1 Study sites

Waimapu, Rangataua and Waikareao sub estuaries were surveyed within Tauranga Harbour, located on the east coast of the north island of New Zealand (-37.62°; 176.04°) in March 2013 (late summer) with samples taken at three different sites within each estuary system (Fig. 4.2). In October 2013 (spring), previous locations were sampled as well as two additional rural areas known as Tuapiro, located in the northern part of Tauranga Harbour and a separate harbour system, Ohiwa Harbour to the east of Tauranga (Fig. 4.2). Sample station locations were selected based on their proximity to likely sources of nutrient contamination (e.g. river entry points) and two more distant locations were included, in order to integrate the range of physical, biological and chemical changes likely to be occurring. In addition, the sites selected within each estuary

have traditionally been used for cultural harvesting of seafood. The estuaries have been further grouped into two general categories based on the degree of human influence. The urban group included Waimapu, Waikareao and Rangataua estuaries. The rural group comprised Tuapiro and Ohiwa estuaries (Chapter 2). Isotope ratios were measured in surface sediment, mangrove (*Avicennia marina*), seagrass (*Zostera marina*), sea lettuce (*Ulva lactuca*), oysters (*Crassostrea gigas*), cockles (*Austrovenus stutchburyi*), mud snails (*Amphibola crenata*) and mud whelks (*Cominella glandiformis*).

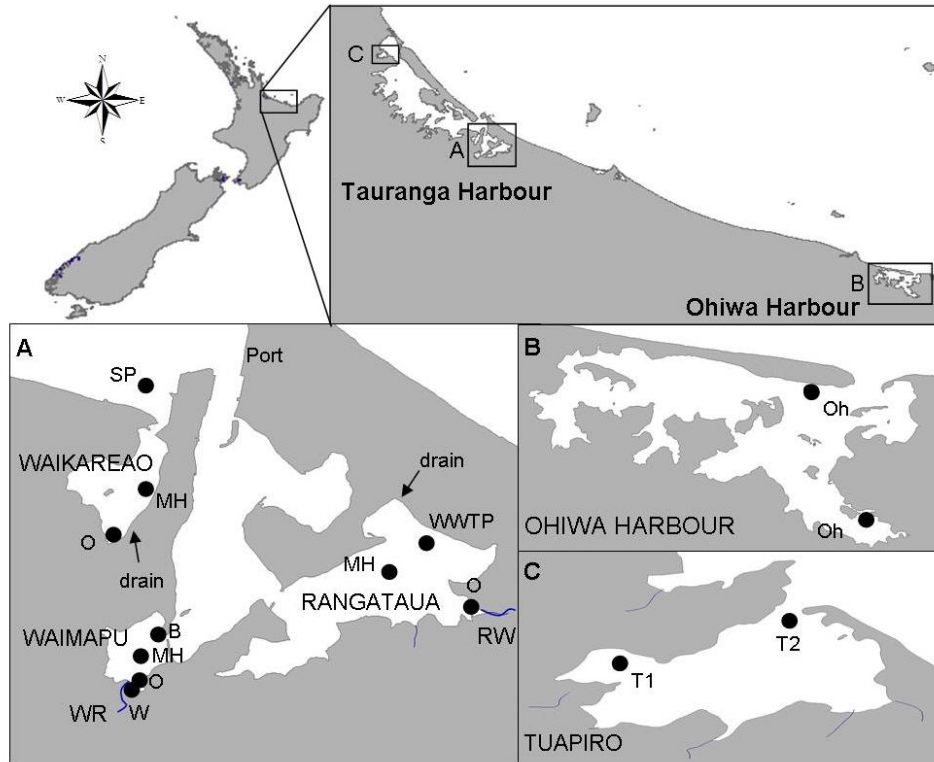


Fig. 4.2. Sampling locations (circles) in Ohiwa and Tauranga Harbours. W: wetland, O: rivers outlet, MH: middle harbour, B: bridge, SP: Sulphur Point, WWTP: wastewater treatment plant, WR: Waimapu River, RW: Waitao River.

An additional (nested) survey was carried out to examine the use of stable isotopes for the detection of treated wastewater seepages in Rangataua estuary in spring (September) and summer (February) 2014 and to correlate patterns of water quality inside and outside the treatment ponds. By collecting a wide range of samples, from both sediments and the associated water, as well as from a

deposit feeder species (mud snails) and a salt tolerant plant (mangrove), the study was designed to provide information on the condition of the environment near to and distant from the WWTP over time through sampling stations across longitudinal transects (Fig. 4.3). Nitrate isotopes were analysed from 14 water samples collected by hand inside Pond 2 and 3 of the WWTP as well as from harbour sites including the drain, the stream, the WWTP Centre and the Middle Harbour sites. A transect starting from close to the potential source of contamination permitted assessment of the intensity of contaminant loading and to estimate the spatial extent of their presence in the estuary.

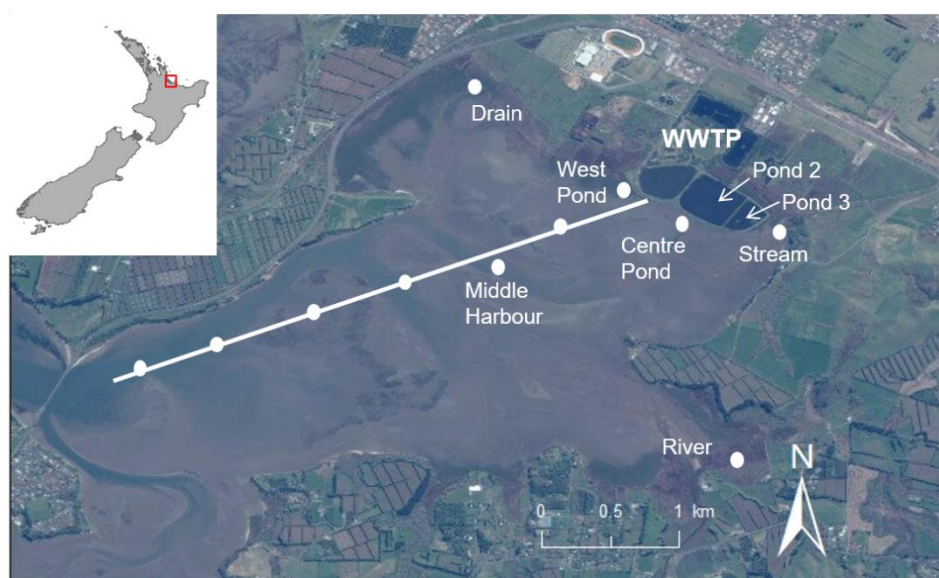


Fig. 4.3. Study area and location of sampling sites (white point) and transect (white line) in Rangataua estuary.

4.2.2 Stable isotopes and elemental analysis

At each site, two replicates of surface sediment were collected from the top 2 cm of the surface with a syringe core. Three subsamples of surface sediments (approximately 10 g per sample) were pooled together to make one composite sample per site. Samples were dried to constant weight in an oven at 50°C. Dry sediment samples were weighed before any laboratory preparation and subsequently sieved to 1 mm and 150 µm to extract rock, pebbles, sand and larger objects. The fine fraction of sediment (<150 µm) was ground to a fine powder

with a mortar and pestle. The sieved portion of sediment was analysed without acidification for the determination of nitrogen ratio, as acidification alters $\delta^{15}\text{N}$ and TN (Lorrain et al., 2003). Subsequent acidification with hydrochloric acid prior to carbon analysis was necessary to remove any inorganic carbon. Sediment was mixed with 1 M HCL and left in the laboratory overnight. Each sample was decanted by adding distilled water and centrifuged four times at 3000 rpm for 4 minutes to remove any acid present. Sediment was re-dried in the oven at 50°C for 30 h and ready for analysis.

Mangrove, seagrass, sea lettuce, cockle, oyster, mud snails and mud whelks were collected by hand from the different locations georeferenced by GPS. Three individuals were pooled and homogenised together to make one composite sample with a minimum of one replicate taken per site. Muscle tissue (foot) and gut samples of cockles, oysters, mud snails and mud whelks were dissected for separate analyses. Biological samples were individually dried in an oven for 3 days at 45°C (to constant weight) and ground to a homogenous powder with a mortar and pestle. Analyses were carried out on a EuroVector 3000 EA system coupled to a GVI Isoprime IRMS for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, total nitrogen (TN) and total carbon (TC).

Isotopic analysis of surface water required the collection of 100 ml per sample and preserved by adding 1 mL of 10% HCl per 100 mL of sample. Samples were analysed following the spongy cadmium method (McIlvin & Altabet, 2005) involving the reduction of nitrate to nitrite with further reduction to nitrous oxide using sodium azide in an acetic acid buffer. Samples were analysed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the nitrate in the water via an Isoprime Tracegas interface using a Gilson 222XL autosampler coupled to a GVI Isoprime IRMS.

Isotope values are reported using δ -notation (‰) as:

$$\delta^{13}\text{C} (\text{‰}) = ((^{13}\text{C}/^{12}\text{C})_{\text{sample}} \div (^{13}\text{C}/^{12}\text{C})_{\text{standard}} - 1) \times 1000$$

$$\delta^{15}\text{N} (\text{‰}) = ((^{15}\text{N}/^{14}\text{N})_{\text{sample}} \div (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1) \times 1000$$

$$\delta^{18}\text{O} (\text{‰}) = ((^{18}\text{O}/^{16}\text{O})_{\text{sample}} \div (^{18}\text{O}/^{16}\text{O})_{\text{standard}} - 1) \times 1000$$

where the international standard was Pee Dee Belemnite (VPDB) for carbon, atmospheric N₂ for nitrogen and carbon and Vienna Standard Mean Ocean Water (VSMOW) for oxygen. Total phosphorus was analysed following the method described in Chapter 2.

4.2.3 Statistical analysis

One-way ANOVA and least significant difference (LSD) post-hoc procedures were used to test differences of nitrogen and phosphorus content in surface sediment between estuaries using Statistica (version 12, Statsoft Inc). PERMANOVA was run on the entire set of data for nitrogen and phosphorus content in surface sediments and species', to examine the significance of the variation between 'season' as well as among 'species' (Primer 6, Primer-E Ltd). PCA is used to simplify the data and to identify the factors that explain most of the variance in the data. PERMANOVA was additionally used when exploring TN and TP concentration in surface sediment between estuaries and different sub areas such as outlet, middle harbour, stormwater drain and WWTP.

4.3 Results

4.3.1 The use of elemental content and stable isotopes to examine food webs and nutrient requirements

The food webs in the Tauranga Harbour displayed variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values specific to species (Fig. 4.4). Mangroves had a lower $\delta^{13}\text{C}$ value (-

26‰) compared to sediment (-23‰), oyster tissues (-21‰), cockle tissues (-19‰), seagrass (-17‰), mud snails tissues (-14‰), sea lettuce (-13‰) and mud whelks (-12‰). $\delta^{13}\text{C}$ values in sea lettuce suggested that the macroalgae was an important food source for mud snails and mud whelks. Muscle $\delta^{13}\text{C}$ was 1.3‰ greater than gut tissue for oysters and mud snails, and ~0.4‰ for cockles. Larger variations of $\delta^{13}\text{C}$ reflected by large standard errors were measured for seagrass and mud whelks. $\delta^{15}\text{N}$ values were lowest for surface sediment (5.4‰) compared to seagrass (5.8‰), mangrove (6.6‰), oyster tissues (6.8‰), sea lettuce (7.8‰), and tissues of cockles (8‰), mud snails (8.6‰) and mud whelks (12‰). For all invertebrates, the $\delta^{15}\text{N}$ values measured in the gut were less than the muscle tissues by 1.3‰ for oyster, 0.6‰ for cockle, 1‰ for mud snails and 1.2‰ for mud whelks. Primary producers (mangrove, seagrass and sea lettuce) and primary consumers (cockles, oysters and mud snails) had lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than secondary consumer (mud whelks), suggesting that mud whelk were the top predator (carnivorous) for the species in the food web studied.

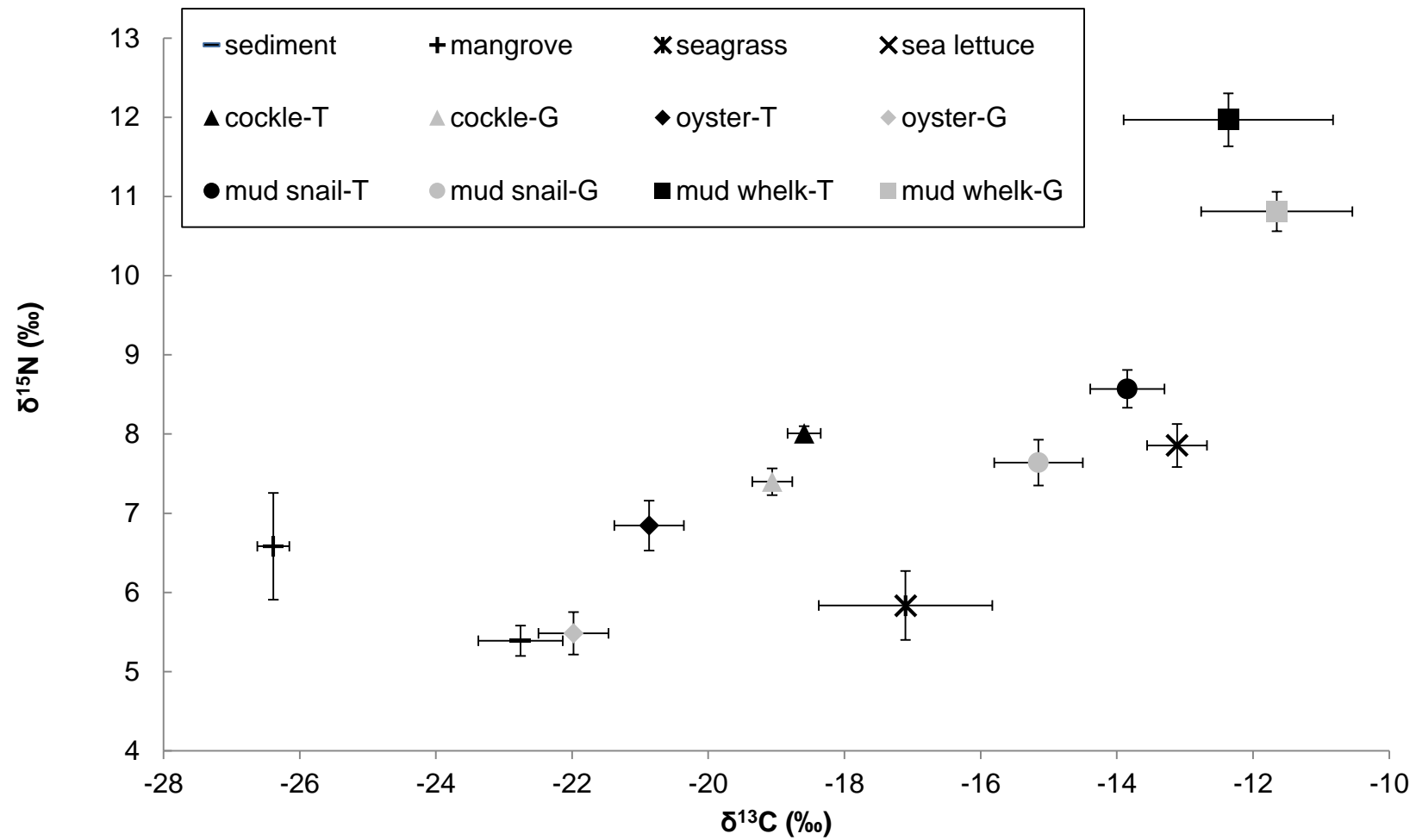


Fig. 4.4. Food web dynamics of some consumers and producers from Tauranga Harbour, T: muscle, G: gut, error bar as standard error.

Concentrations of C, N and P varied between species in Tauranga Harbour (Table 4.1). In the present study, a mean value of C:N:P atomic ratios of 19:2:1 was obtained for surface sediment, 273:19:1 for mangrove, 223:16:1 for seagrass, 309:27:1 for sea lettuce, 64:15:1 for cockles, 56:12:1 for oysters, 90:22:1 and 83:20:1 for mud snails and mud whelks respectively. C content was higher in mangrove (44%) than seagrass, oysters and mud whelks (~40%), cockles and sea lettuce (~35%), mud snails (29%) and surface sediment (1.9%). N content was higher in mud whelks (9.4%) than cockles and oysters (8.4%), mud snails (7.2%), mangrove, sea grass and sea lettuce (3%) and sediment (0.2%). P content was higher in oysters (0.7%) than cockles (0.6%), mud whelks (0.5%), mud snails (0.4%), mangrove and seagrass (0.2%) and sea lettuce and sediment (0.1%) exhibiting increasingly lower P content.

Table 4.1. Mean \pm SE of the total carbon (TC), nitrogen (TN) and phosphorus (P) content and the C:N, C:P and N:P ratio of the entire set of samples of sediment, mangrove, seagrass, sea lettuce, cockles, oysters, mud snails and mud whelks in Tauranga Harbour.

| Species | TC (%) | TN (%) | P (%) | C:N | C:P | N:P |
|-------------|-----------------|----------------|-----------------|--------------|----------------|--------------|
| Sediment | 1.9 \pm 0.29 | 0.2 \pm 0.02 | 0.13 \pm 0.01 | 12 \pm 1.4 | 19 \pm 3.0 | 2 \pm 0.3 |
| Mangrove | 44.2 \pm 0.99 | 3.0 \pm 0.45 | 0.17 \pm 0.01 | 17 \pm 1.0 | 273 \pm 13.7 | 19 \pm 3.4 |
| Seagrass | 38.1 \pm 0.66 | 2.8 \pm 0.10 | 0.19 \pm 0.01 | 14 \pm 0.4 | 223 \pm 12.9 | 16 \pm 0.7 |
| Sea lettuce | 33.6 \pm 0.80 | 3.0 \pm 0.13 | 0.12 \pm 0.01 | 11 \pm 0.3 | 309 \pm 25.9 | 27 \pm 2.1 |
| Cockle | 35.2 \pm 0.56 | 8.4 \pm 0.16 | 0.61 \pm 0.06 | 4 \pm 0.0 | 64 \pm 4.8 | 15 \pm 1.1 |
| Oyster | 40.7 \pm 0.63 | 8.4 \pm 0.28 | 0.73 \pm 0.02 | 5 \pm 0.2 | 56 \pm 2.1 | 12 \pm 0.5 |
| Mud snail | 29.3 \pm 1.34 | 7.2 \pm 0.39 | 0.35 \pm 0.02 | 4 \pm 0.1 | 90 \pm 6.7 | 22 \pm 1.9 |
| Mud whelk | 38.7 \pm 1.46 | 9.4 \pm 0.52 | 0.47 \pm 0.02 | 4 \pm 0.1 | 83 \pm 0.2 | 20 \pm 0.3 |

TC, TN and TP in mangrove, sea lettuce and sea grass collected from the Tauranga Harbour were higher in winter than summer (Fig. 4.5). Significance has been tested with PERMANOVA examining two factors and their mutual interactions: 'Species' and 'Season' (Table 4.2). Results were statistically different between summer and winter for all species. Seasonal variation was consistent across 'species' for TC, with higher TC found in mangrove than seagrass and sea lettuce (minimum values in sea lettuce). Comparison between different species showed that TN content was higher in mangrove in winter and in sea lettuce in summer. Mangrove was the most seasonally affected species with a lower TN in summer. TP was higher in seagrass in winter (0.21%) and in mangrove and seagrass in summer (0.13%). N:P and C:P showed similar fluctuations with most variation found in sea lettuce, with higher C:P and N:P ratio measured in summer. Seasonal variations of C:P and N:P were smaller in mangrove and seagrass following similar fluctuation than TC. Seasonal variation of C:N were small but higher in mangrove with higher values found in summer.

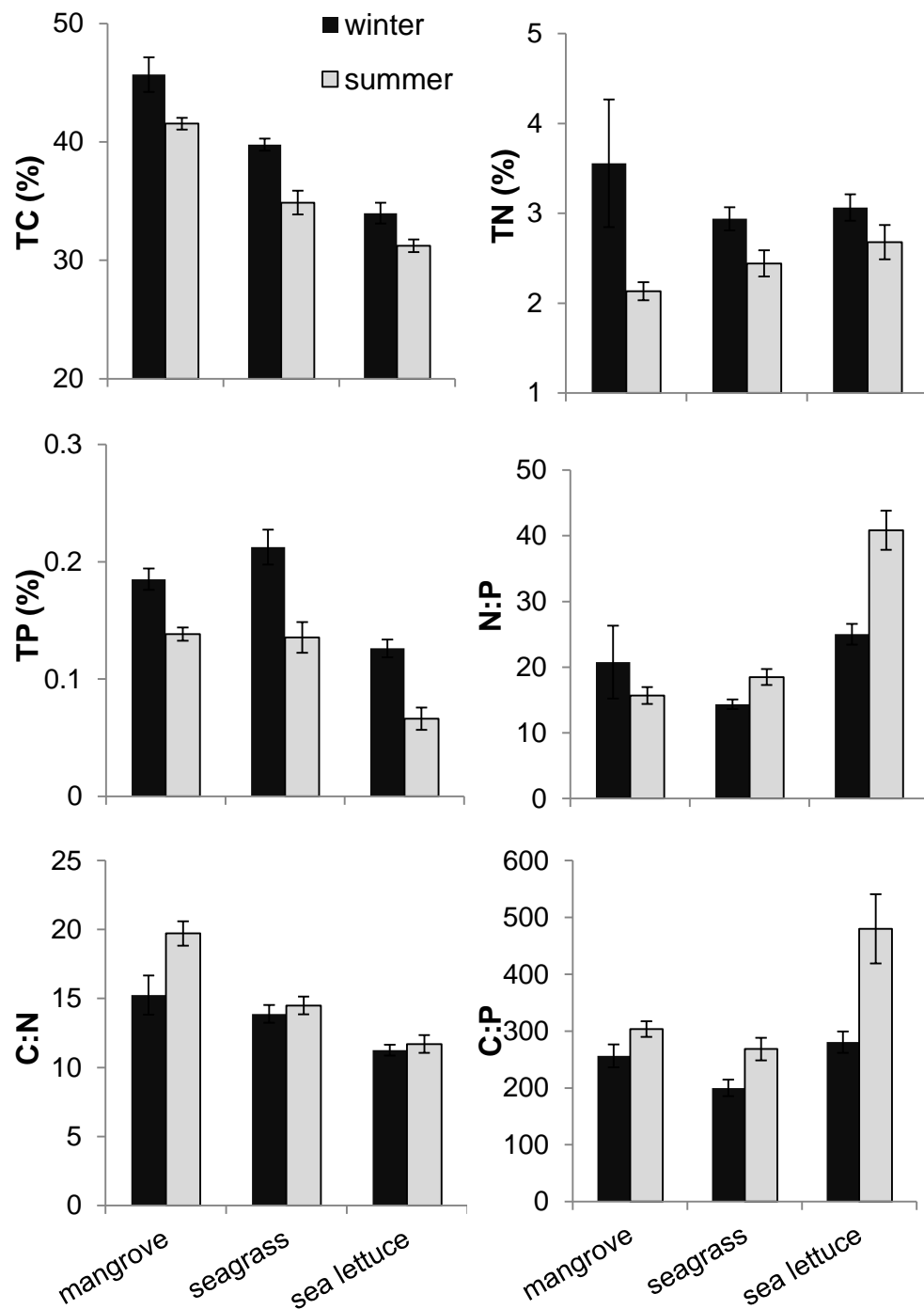


Fig. 4.5. Seasonal variations (average of all sampling sites) in carbon, nitrogen and phosphorus (%) content and the N:P, C:P and C:N atomic ratio in mangrove, seagrass and sea lettuce, error bars as standard error.

Table 4.2. Results of the PERMANOVA analysis for TN, TC, TP content and N:P, C:N, C:P comparing species (mangrove, seagrass, sea lettuce) and season; df: degree of freedom, SS: sum of squares, MS: mean sum of squares.

| PERMANOVA | df | SS | MS | Pseudo- F | P (perm) |
|----------------------------------|------|--------|-------|--------------|-------------|
| Species | 1 | 55.32 | 55.32 | 22.65 | 0.001 |
| Season | 2 | 111.16 | 55.58 | 22.78 | 0.001 |
| Species \times Season | 2 | 12.308 | 6.15 | 2.52 | 0.033 |
| Residual | 44 | 107.47 | 2.44 | | |
| Total | 49 | 294 | | | |
| Pair-wise test " <u>summer</u> " | t | P | | | |
| | | (perm) | | | |
| Mangrove \times seagrass | 3.15 | 0.012 | | | |
| mangrove \times sea lettuce | 5.83 | 0.001 | | | |
| seagrass \times sea lettuce | 3.86 | 0.001 | | | |
| Pair-wise test " <u>winter</u> " | t | P | | | |
| | | (perm) | | | |
| mangrove \times seagrass | 3.48 | 0.004 | | | |
| mangrove \times sea lettuce | 5.79 | 0.024 | | | |
| seagrass \times sea lettuce | 5.09 | 0.028 | | | |

Principal component analysis (PCA) plots of data from sea grass, mangrove, algae and invertebrates within Tauranga harbour showed that PC1 and PC2 explained 89% of total variation. Two distinct groups could be identified associated with nutrient accumulation and distinct nutrient physiology requirements (Fig 4.6). The first group included mangrove, seagrass and sea lettuce together and was associated with higher carbon content. The second group included all invertebrates and was associated with higher TP and TN. Mud snails showed more variation in TP and N:P than other species.

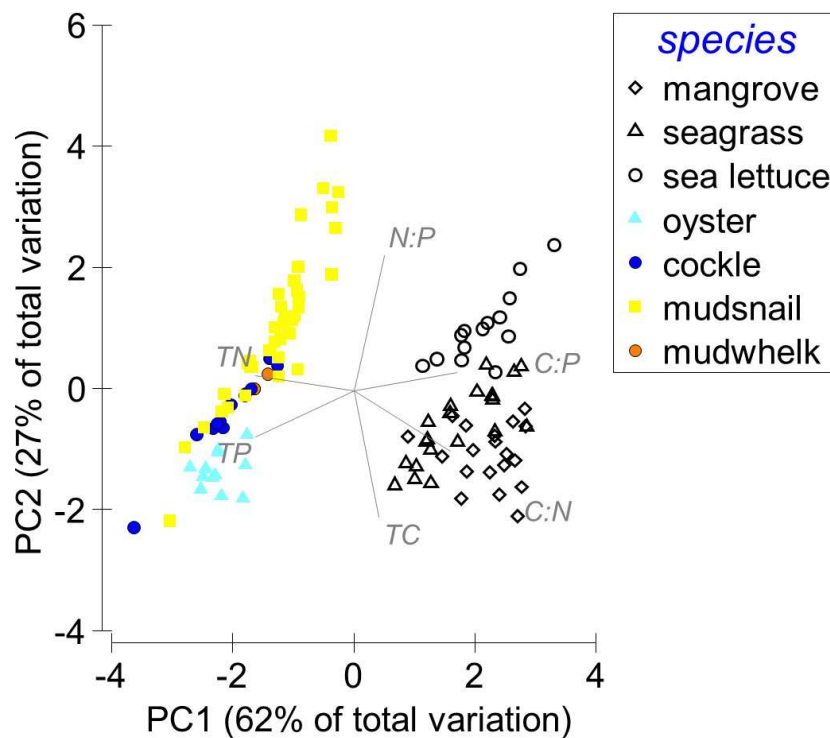


Fig. 4.6. Principal component analysis of nitrogen, phosphorus and carbon content and the N:P, C:P and C:N ratio in mangrove, seagrass, sea lettuce, oysters, cockles, mud snails and mud whelks

4.3.2 The use of stable isotopes to detect anthropogenic inputs

Bay of Plenty Estuaries

Total nitrogen and phosphorus in surface sediment displayed different variations with locations (Fig. 4.7). Total phosphorus was higher in Rangataua (0.21%) than Waimapu (0.16%) and Waikareao (0.15%). The lowest concentration of P was measured in the rural estuaries (0.1%). TN was higher in

Waimapu (0.29%) than Rangataua (0.13%) and Waikareao (0.14%) with lowest concentration found in rural estuaries (0.09%). Total nitrogen in Waimapu was significantly higher than in the other sampling locations ($F_{(3,29)}=3.69$; $P=0.02$; post hoc LSD test, $P<0.01$).

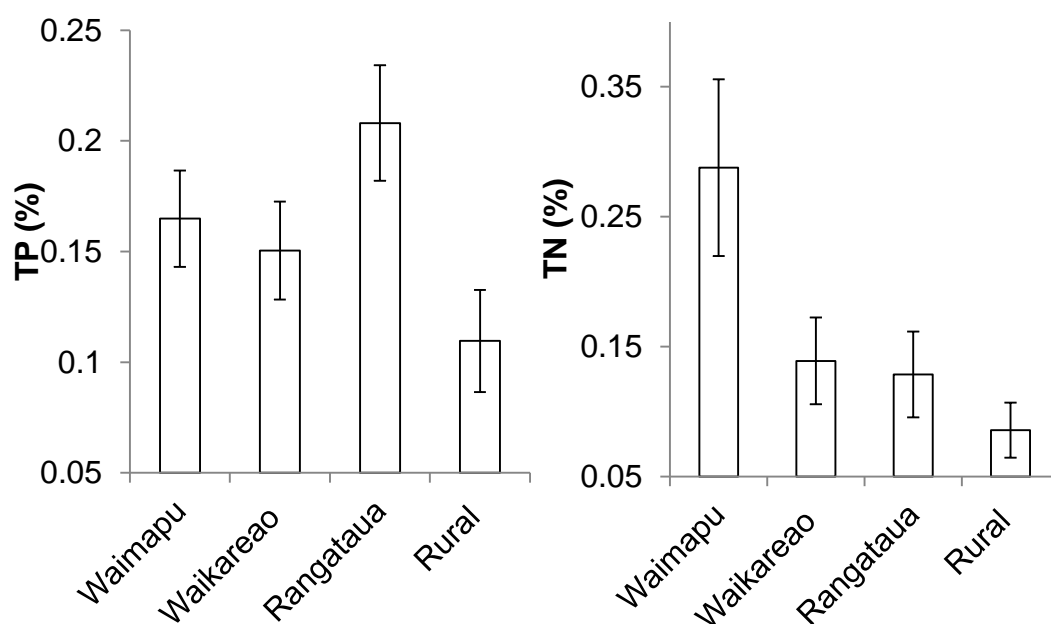


Fig. 4.7. Total nitrogen (TN) and total phosphorus (TP) measured in surface sediment in Waimapu, Waikareao, Rangataua and rural estuaries.

TN and TP in urban and rural estuaries were compared with data from other estuaries showing higher concentrations of phosphorus in Tauranga Harbour (Table 4.3). TP in Tauranga Harbour was higher than concentrations found in other studies: e.g China, USA, Italy and Brazil. TP in Ohiwa Harbour had similar concentrations that the Pearl River estuary in China. TN in Waimapu was lower than in Chesapeake Bay, USA but higher than any other estuaries. TN measured in Rangataua, Waikareao and Tuapiro had similar values to sediment in Italy. However, Ohiwa Harbour displayed a lower level of nitrogen than any other estuaries.

Table 4.3. Total nitrogen (%) and total phosphorus (%) in surface sediment in Tauranga and Ohiwa Harbour and worldwide estuaries

| | TN | TP | References |
|----------------------------------|-----------|--------|---|
| Waimapu, Tauranga Harbour | 0.29 | 0.16 | this study |
| Rangataua, Tauranga Harbour | 0.13 | 0.21 | this study |
| Waikareao, Tauranga Harbour | 0.14 | 0.15 | this study |
| Tuapiro, Tauranga Harbour | 0.13 | 0.14 | this study |
| Ohiwa Harbour | 0.06 | 0.09 | this study |
| Pearl River Estuary, China | 0.16 | 0.08 | Wang et al. 2015b |
| Chesapeake Bay, USA | 0.33-0.47 | 0.057 | Zimmerman and Canuel. 2000; Bratton et al. 2003 |
| Western Mediterranean Sea, Italy | 0.13 | 0.07 | Porrello et al. 2005 |
| Paraguacu estuary, Brazil | <0.22% | ~0.038 | Pereira et al. 2015 |

TN concentration in surface sediment showed important variations between different locations within the same estuary (Fig. 4.8). In Waimapu, higher concentrations of TN were found in the wetland (0.48%). Waimapu, Waikareao and Rangataua showed higher content of nitrogen at the outlets of rivers and drains (0.14-0.27%). For the rural area, TN was lower in Ohiwa (0.06%) than Tuapiro (0.13%). TP concentration in surface sediment displayed strong variation within the same estuary with different fluctuations with locations than TN (Fig. 4.9). In Waimapu, most of the phosphorus was present in the wetland (0.25%). In Waikareao, higher TP was measured at the outlet of the drain (0.21%) as well as the sulphur point area (0.17%). TP in Rangataua was present in significant concentration for all sites, with higher concentrations recorded around the WWTP (0.28%). TP in Tuapiro was similar to the middle harbour of Waimapu (0.14%); however Ohiwa recorded a lower concentration of P (0.09%). PERMANOVA indicated no significance difference between estuaries indicating similarities between the level of contaminations (Table 4.4), however significant differences were found for some sub-areas within the same estuary. $\delta^{15}\text{N}$ values were higher around the WWTP (8.4‰) and Sulphur Point (6.5‰). Rivers, middle harbour and drains had similar $\delta^{15}\text{N}$ values (4.3-5.5‰).

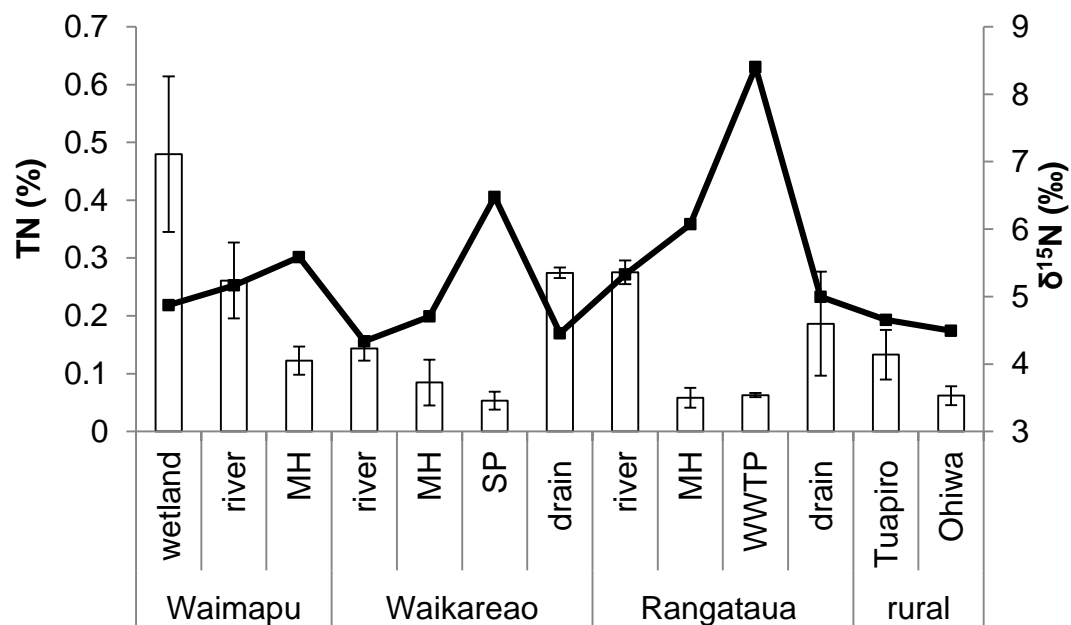


Fig. 4.8. Total nitrogen content (bars) and $\delta^{15}\text{N}$ values (solid line) in surface sediment collected from Bay of Plenty estuaries

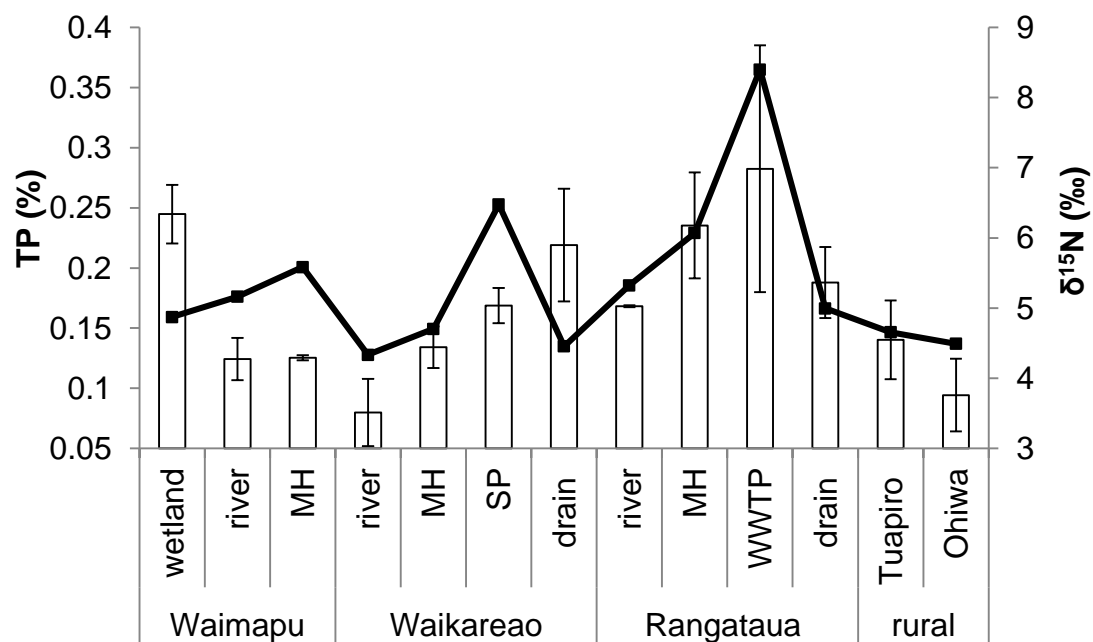


Fig. 4.9. Total phosphorus content (bars) and $\delta^{15}\text{N}$ values (solid line) in surface sediment collected from Bay of Plenty estuaries.

Table 4.4. Results of the PERMANOVA analysis for TN, TP in sediment comparing estuaries and areas; df: degree of freedom, SS: sum of squares, MS: mean sum of squares.

| PERMANOVA | df | SS | MS | Pseudo- F | P (perm) |
|------------------|------|--------|------|--------------|-------------|
| Estuaries | 2 | 1.72 | 0.86 | 0.86 | 0.482 |
| Area | 5 | 23.89 | 7.78 | 4.79 | 0.002 |
| Residual | 21 | 20.95 | 0.99 | | |
| Total | 32 | 64 | | | |
| Pair wise test | t | P | | | |
| | | (perm) | | | |
| wetland × outlet | 2.4 | 0.045 | | | |
| wetland × MH | 8.95 | 0.006 | | | |
| outlet × MH | 2.43 | 0.004 | | | |
| outlet × SP | 2.1 | 0.031 | | | |
| outlet × drain | 1.93 | 0.068 | | | |
| outlet × WWTP | 1.97 | 0.054 | | | |
| MH × drain | 1.06 | 0.34 | | | |
| MH × WWTP | 1.08 | 0.34 | | | |
| SP × drain | 2.73 | 0.021 | | | |
| drain × WWTP | 1.11 | 0.442 | | | |

Variations of total nitrogen in surface sediments were statistically different for $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$ (Fig. 4.10). Significant increases in total nitrogen in surface sediments with lower $\delta^{13}\text{C}$ values was observed ($R^2=0.53$, $P=0.005$; Fig. 4.10). TN did not show any significance difference with $\delta^{15}\text{N}$. The highest concentration of TN was found at the wetland site (0.5%) with a $\delta^{13}\text{C}$ value of -30.8‰. The lowest TN was found at Sulphur Point (0.05%) with a $\delta^{13}\text{C}$ of -19.3‰.

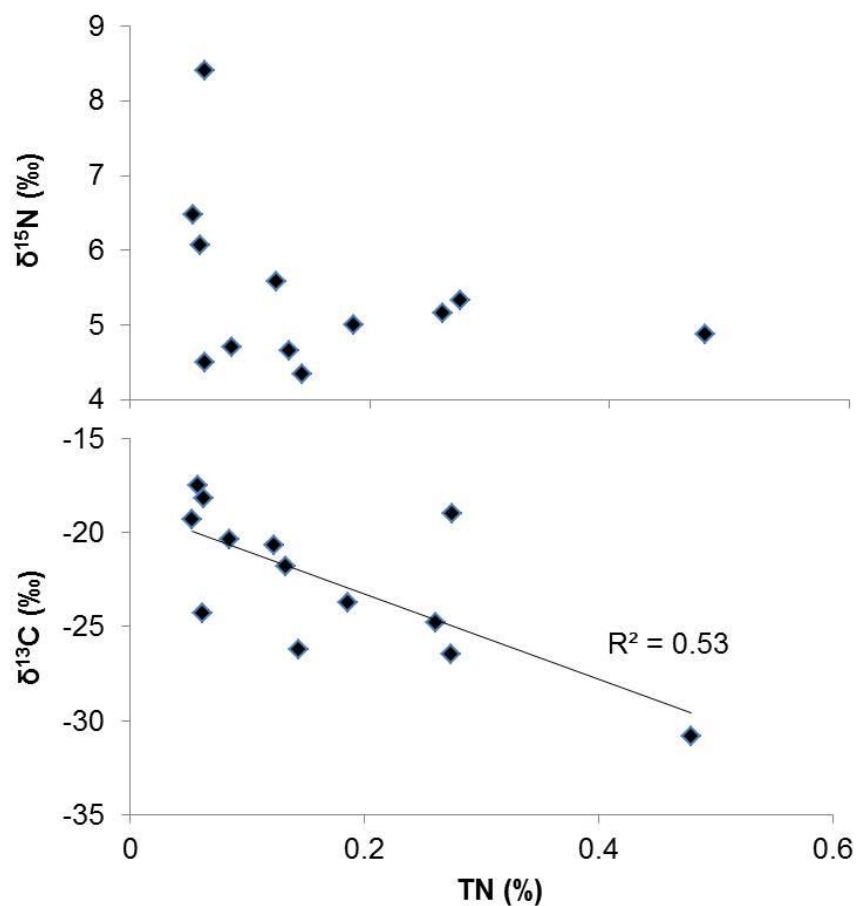


Fig. 4.10. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratio vs total nitrogen in surface sediment collected in Tauranga Harbour.

Variation of total phosphorus significantly increased with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Fig 4.11). Total phosphorus significantly increased with higher $\delta^{15}\text{N}$ ($R^2=0.72$, $P<0.001$) with maximum values found in the proximity of the WWTP. Total phosphorus increased with higher $\delta^{13}\text{C}$ values ($R^2=0.56$, $P<0.001$), with the minimum concentration found at the Waikareao outlet.

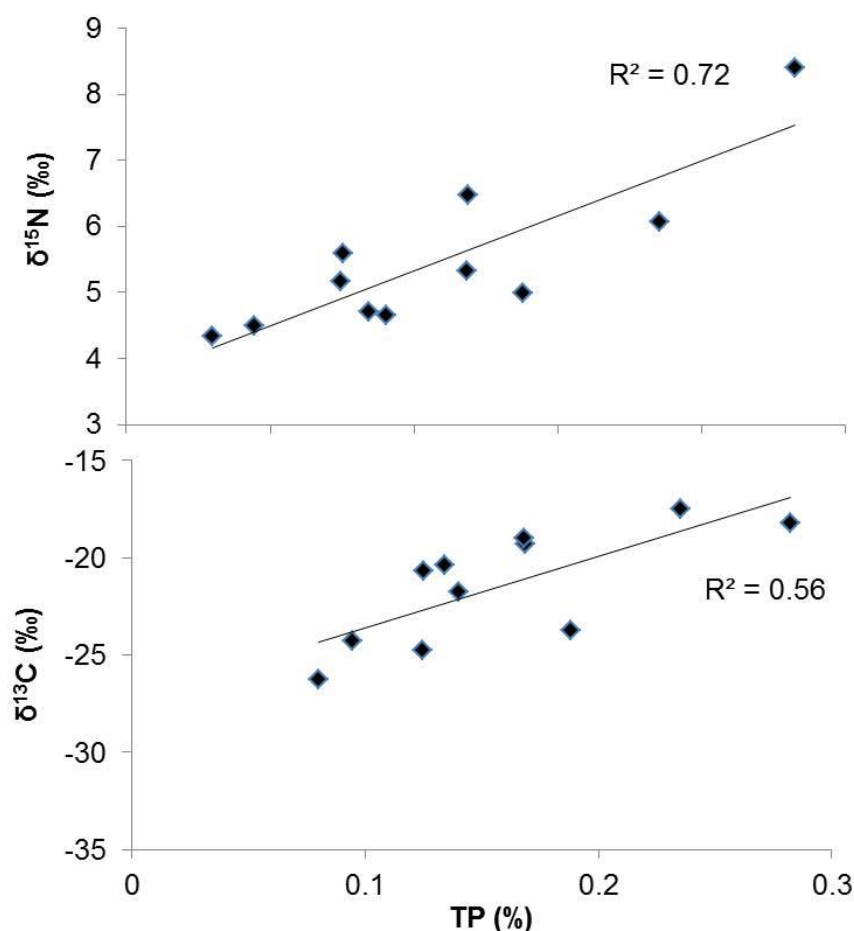


Fig. 4.11. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ vs TP (%) in surface sediment collected in Tauranga Harbour.

4.3.3 Rangataua estuary

A transect from the WWTP to the outer part of the Rangataua estuary revealed spatial and seasonal variability in sedimentary $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, with smaller values generally being observed further away from the WWTP (Fig. 4.12). Sedimentary $\delta^{15}\text{N}$ values near the WWTP displayed no seasonal variation, with consistently higher values of $\sim 9\text{‰}$. In winter, $\delta^{15}\text{N}$ values decreased by up to $\sim 3.5\text{‰}$ with increasing distance from the WWTP (5.5‰ at 500 m from WWTP). This depletion of $\delta^{15}\text{N}$ values with distance, was less marked in summer with values as low as 6.7‰ at around 1 km away from the WWTP. Although $\delta^{15}\text{N}$ values generally became smaller with distance from the WWTP, the sedimentary $\delta^{15}\text{N}$ values were still positive at distances of up to 1.8 km away. In winter, greater $\delta^{15}\text{N}$ values were measured from 500 m to 1800 m ($+2.9\text{‰}$). This enrichment of

sedimentary $\delta^{15}\text{N}$ was more marked than summer (+0.4‰). Finally, from 1.8 km to 3.0 km, the effect of the WWTP on sedimentary $\delta^{15}\text{N}$ values had weakened. Nevertheless, greater $\delta^{15}\text{N}$ values in winter relative to the summer were still observed, even at this distance, with winter values being higher (+1.6‰) than summer (-1.4‰). Sedimentary $\delta^{13}\text{C}$ values revealed the same broad trend as $\delta^{15}\text{N}$. $\delta^{13}\text{C}$ values near the WWTP displayed no seasonal variation, with consistently greater values of ~-16‰, providing an additional line of evidence supporting the enriched $\delta^{15}\text{N}$ values of treated sewage signal. In winter and summer, $\delta^{13}\text{C}$ values decreased by up to ~3‰ with increasing distance from the WWTP (-19 to -20‰ at 3 km away from WWTP). Although $\delta^{13}\text{C}$ values generally decreased consistently with distance from the WWTP, sedimentary $\delta^{13}\text{C}$ values were lower at one site localised at 1000 m away of the WWTP in winter(-21.4‰), while less marked in summer (-17.9‰).

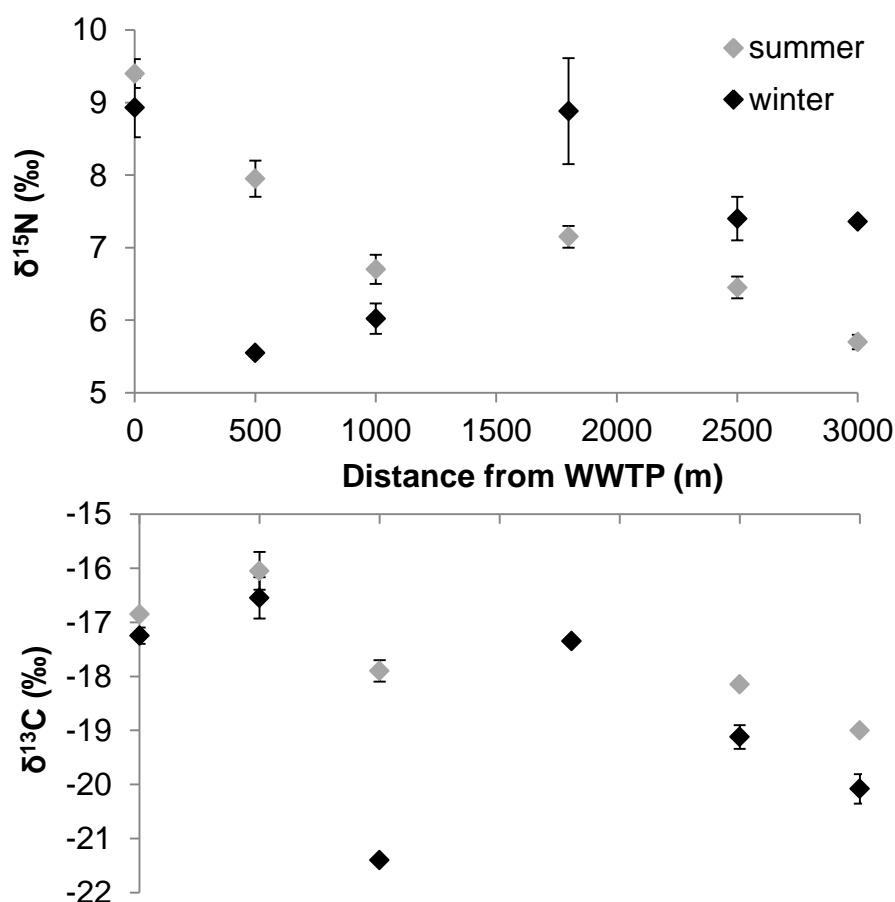


Fig. 4.12. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in surface sediment as a function of downstream distance (m) from the wastewater treatment plant.

$\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ in surface water displayed seasonal and spatial variation with clear segregation between samples collected within the oxidation ponds and others collected directly from the Rangataua Estuary (Fig. 4.13). $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ values in pond 2 displayed more seasonal variation than pond 3. Pond 2 displayed greater $\delta^{15}\text{N}\text{-NO}_3$ values than pond 3 by +3‰ in summer and +8‰ in winter. $\delta^{15}\text{N}\text{-NO}_3$ values in winter were higher than summer in pond 2 (+4‰) and slightly less for pond 3 (-0.8‰). $\delta^{18}\text{O}\text{-NO}_3$ values in winter were higher in pond 2 (+9‰) and pond 3 (+4‰). Water samples collected in the Rangataua Estuary displayed seasonal and spatial variation. In summer and winter, smaller $\delta^{15}\text{N}\text{-NO}_3$ values were found near the drain and stream (~6‰) and higher values recorded in the centre pond (~9-10‰). $\delta^{18}\text{O}\text{-NO}_3$ values displayed a wide range of values from 0.9‰ at the drain site to 11‰ at the middle harbour site in

summer, and from 1.5‰ at the stream to 15‰ for the middle harbour site. Seasonal variation in $\delta^{18}\text{O}-\text{NO}_3$ values were small for sites close to point sources such as the drain and the stream.

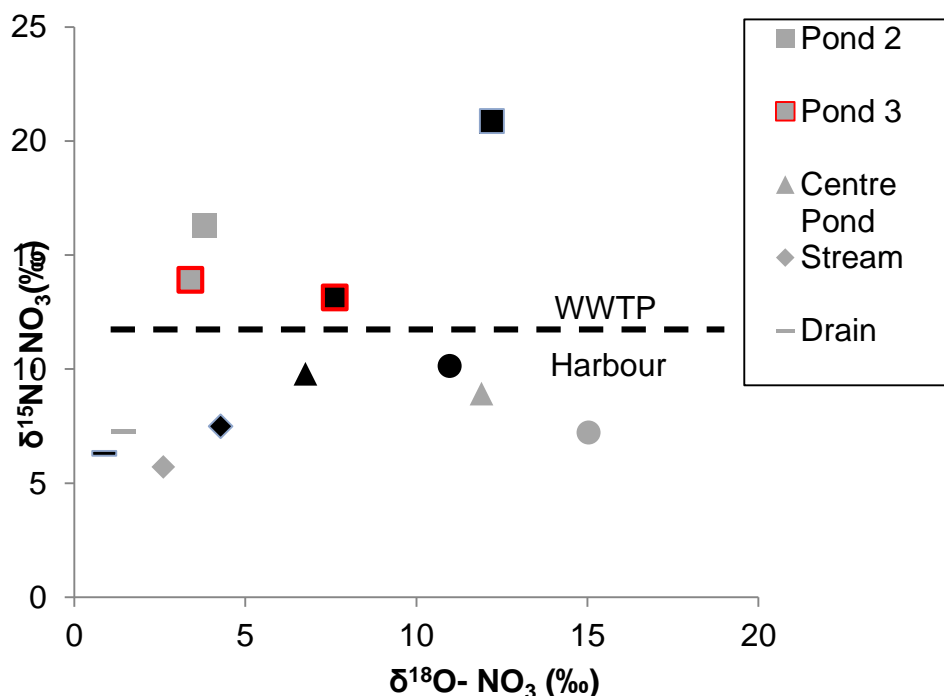


Fig. 4.13. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in nitrate of surface water collected in wastewater ponds and Rangataua estuary water in summer (grey) and winter (black).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in surface sediment displayed variation for different sites and season within the Rangataua Estuary (Table 4.5). Sedimentary $\delta^{15}\text{N}$ values were significantly higher in summer (T-test; $P < 0.01$) than winter. At the middle harbour, $\delta^{13}\text{C}$ values in summer were higher by +5‰ compared to winter possibly due to an increase of sea lettuce in summer with higher $\delta^{13}\text{C}$ values (~-13‰) and potential decomposition on the top of the sediment. While more evident for $\delta^{15}\text{N}$, rather than $\delta^{13}\text{C}$, isotopic ratios in sediment were greater at nearest location of the WWTP (west pond) in summer and winter by +3‰ for nitrogen when compared to the site furthest away (middle harbour). Sedimentary $\delta^{13}\text{C}$ was similar in summer between these two sites, however, sedimentary $\delta^{13}\text{C}$ values in winter were higher in west ponds by +4‰ when compared to the middle harbour site. Lower values of $\delta^{15}\text{N}$ (~5‰) and $\delta^{13}\text{C}$ (-21‰) were found at the drain in

summer and winter. At the drain, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were different to other sites. $\delta^{13}\text{C}$ at the Centre pond was higher in summer and winter ($\sim -18\text{‰}$) with important variation of $\delta^{15}\text{N}$ between summer and winter. In summer, $\delta^{15}\text{N}$ values were greater (7.80‰) but -2‰ less in winter.

Table 4.5. Mean (\pm S.E.) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in surface sediment in Rangataua Estuary in summer and winter.

| sediment | Summer | | Winter | |
|----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Site | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ |
| River | 5.94 ± 0.17 | -23.47 ± 0.35 | 5.32 ± 0.18 | -19.05 ± 0.78 |
| Middle harbour | 6.65 ± 1.05 | -16.40 ± 0.10 | 6.02 ± 0.21 | -21.40 ± 0.05 |
| Drain | 5.57 ± 0.17 | -21.20 ± 0.70 | 5.02 ± 0.02 | -21.88 ± 0.12 |
| Stream | 7.80 ± 0.60 | -19.25 ± 0.45 | 6.44 ± 0.01 | -22.66 ± 0.72 |
| West pond | 9.40 ± 0.20 | -16.85 ± 0.05 | 8.93 ± 0.41 | -17.25 ± 0.15 |
| Centre pond | 7.80 ± 0.90 | -17.25 ± 0.55 | 5.70 ± 0.06 | -18.06 ± 0.06 |

Total nitrogen content in surface sediment was higher at the river (0.27%) with no seasonal variation (Fig. 4.14). The middle harbour site had the lowest nitrogen content (0.06%) in summer and winter with small seasonal fluctuation. The drain site showed higher nitrogen content in winter (0.19%) than summer (0.08%). The WWTP site had higher nitrogen content in summer (0.1%) than winter (0.06%). PERMANOVA analysis showed no significance difference between drain and WWTP, drain and Middle Harbour, WWTP and Middle Harbour which emphasised on widespread levels of contamination of TN and TP in the estuary (Table 4.6).

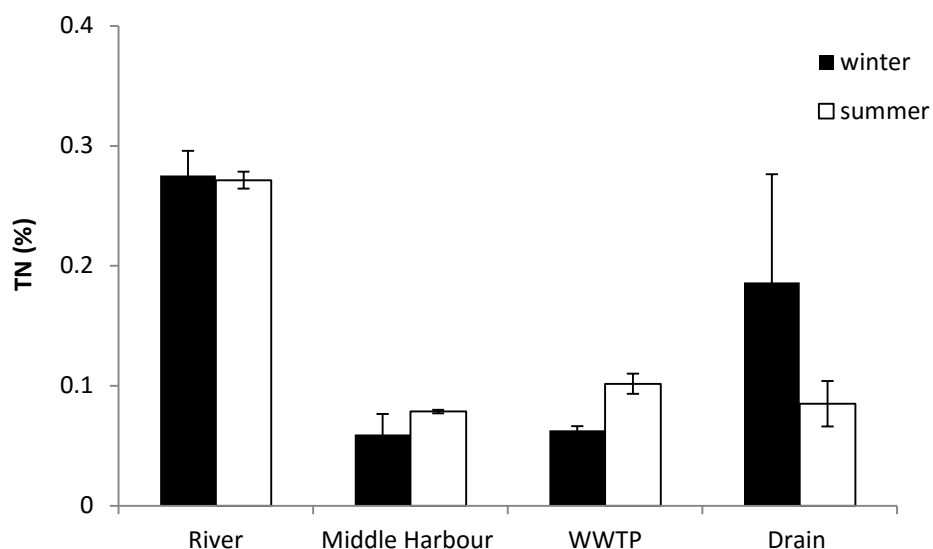


Fig. 4.14. Seasonal nitrogen content (%) in surface sediment collected in Rangataua estuary

Total phosphorus in surface sediment was higher in winter for all sites with important reduction of TP in summer (Fig. 4.15). In winter, the WWTP had the highest TP concentration (0.28%) which was lower in summer (0.03%). In summer, higher concentration of TP was found at the drain (0.07%).

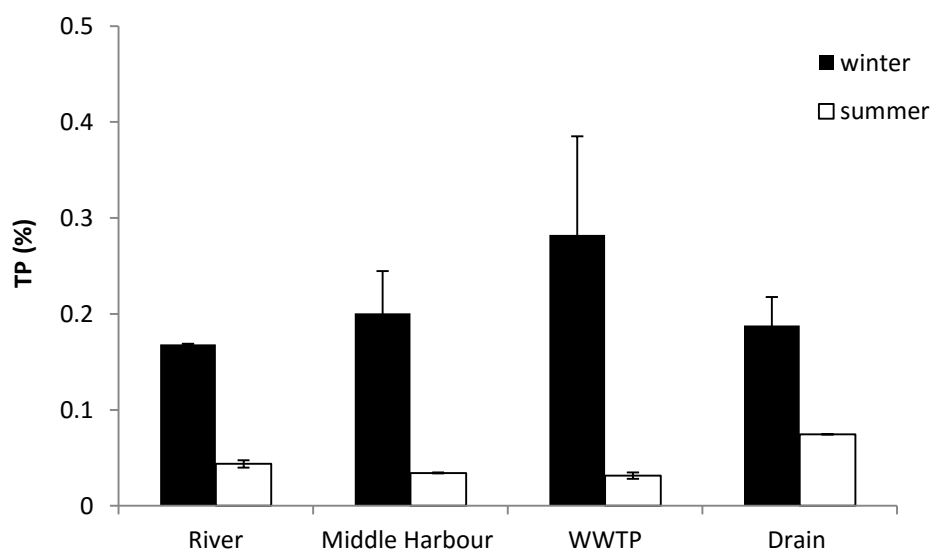


Fig. 4.15. Seasonal nitrogen and phosphorus content (%) in surface sediment collected in Rangataua estuary.

Table 4.6. Results of the PERMANOVA analysis for TN, TP in sediment comparing season and areas; df: degree of freedom, SS: sum of squares, MS: mean sum of squares.

| PERMANOVA | df | SS | MS | Pseudo-F | P (perm) |
|----------------------|------|--------|-------|----------|----------|
| Season | 1 | 12.89 | 12.89 | 22.34 | 0.001 |
| Area | 3 | 16.41 | 5.47 | 9.48 | 0.001 |
| Area \times season | 3 | 2.36 | 0.79 | 1.36 | 0.26 |
| Residual | 13 | 7.50 | 0.58 | | |
| Total | 20 | 40.00 | | | |
| Pair wise test | T | P | | | |
| | | (perm) | | | |
| Drain \times WWTP | 1.12 | 0.283 | | | |
| Drain \times MH | 1.65 | 0.072 | | | |
| Drain \times river | 2.58 | 0.019 | | | |
| WWTP \times MH | 1.00 | 0.358 | | | |
| WWTP \times river | 4.89 | 0.001 | | | |
| MH \times river | 6.47 | 0.001 | | | |

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in muscle tissues of mud snails displayed variations for different locations and season (Table 4.7). In the river, $\delta^{15}\text{N}$ values in tissues were similar in summer and winter while $\delta^{13}\text{C}$ values were higher by +3‰ in winter. For the river, higher $\delta^{13}\text{C}$ values in surface sediment were found in winter which could explain the seasonal fluctuation of $\delta^{13}\text{C}$ values in mud snails. Mud snails collected near the drain had smaller $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in tissues (7.8‰ and ~-17‰ respectively) than the middle harbour (~8.5‰ and -11‰), with no seasonal variation possibly due to a constant source of contamination. This confirmed previous results in sediment with no seasonal variation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. At the stream, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in tissues were higher compared to the middle harbour in summer and winter. $\delta^{15}\text{N}$ in tissues of mud snails were higher in winter by +2‰ while smaller by -3‰ for $\delta^{13}\text{C}$. At the west pond, higher values of $\delta^{15}\text{N}$ (10.6‰) and $\delta^{13}\text{C}$ (-10.6‰) were found in summer and winter

most likely due to the leakage of treated wastewater. Finally, tissues at the Centre pond had higher and similar $\delta^{15}\text{N}$ (10‰) and $\delta^{13}\text{C}$ (-10‰) values in summer and winter than middle harbour most likely due to its proximity to the wastewater ponds.

Table 4.7. Mean (\pm S.E.) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in mud snails in Rangataua estuary in summer and winter.

| Site | <i>Summer</i> | | <i>Winter</i> | |
|----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ |
| Outlet | 8.03 \pm 0.02 | -18.25 \pm 0.36 | 8.32 \pm 0.14 | -15.79 \pm 0.14 |
| Middle Harbour | 8.71 \pm 0.09 | -11.20 \pm 0.02 | 8.44 \pm 0.00 | -11.04 \pm 0.08 |
| Drain | 7.80 \pm 0.12 | -17.19 \pm 0.03 | 7.86 \pm 0.18 | -16.88 \pm 0.38 |
| Stream | 9.85 \pm 0.11 | -9.44 \pm 0.45 | 11.44 \pm 0.14 | -12.72 \pm 0.42 |
| West Pond | 10.58 \pm 0.10 | -10.64 \pm 0.26 | 11.25 \pm 0.31 | -11.95 \pm 0.30 |
| Center Pond | 9.97 \pm 0.09 | -10.08 \pm 0.09 | 9.83 \pm 0.05 | -10.66 \pm 0.02 |

Linear regression in $\delta^{15}\text{N}$ values of tissues ($R^2=0.72$, $P=0.001$), and gut ($R^2=0.60$, $P=0.008$) of mud snails in summer showed a strong correlation with surface sediment (Fig. 4.16A). In winter, linear regression showed weaker correlation for tissues ($R^2=0.48$, $P=0.03$) but stronger for the gut content ($R^2=0.70$, $P=0.002$) as showed in Fig. 16B. Generally, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values found in the gut were smaller by ~1-2‰ compared to muscle tissues.

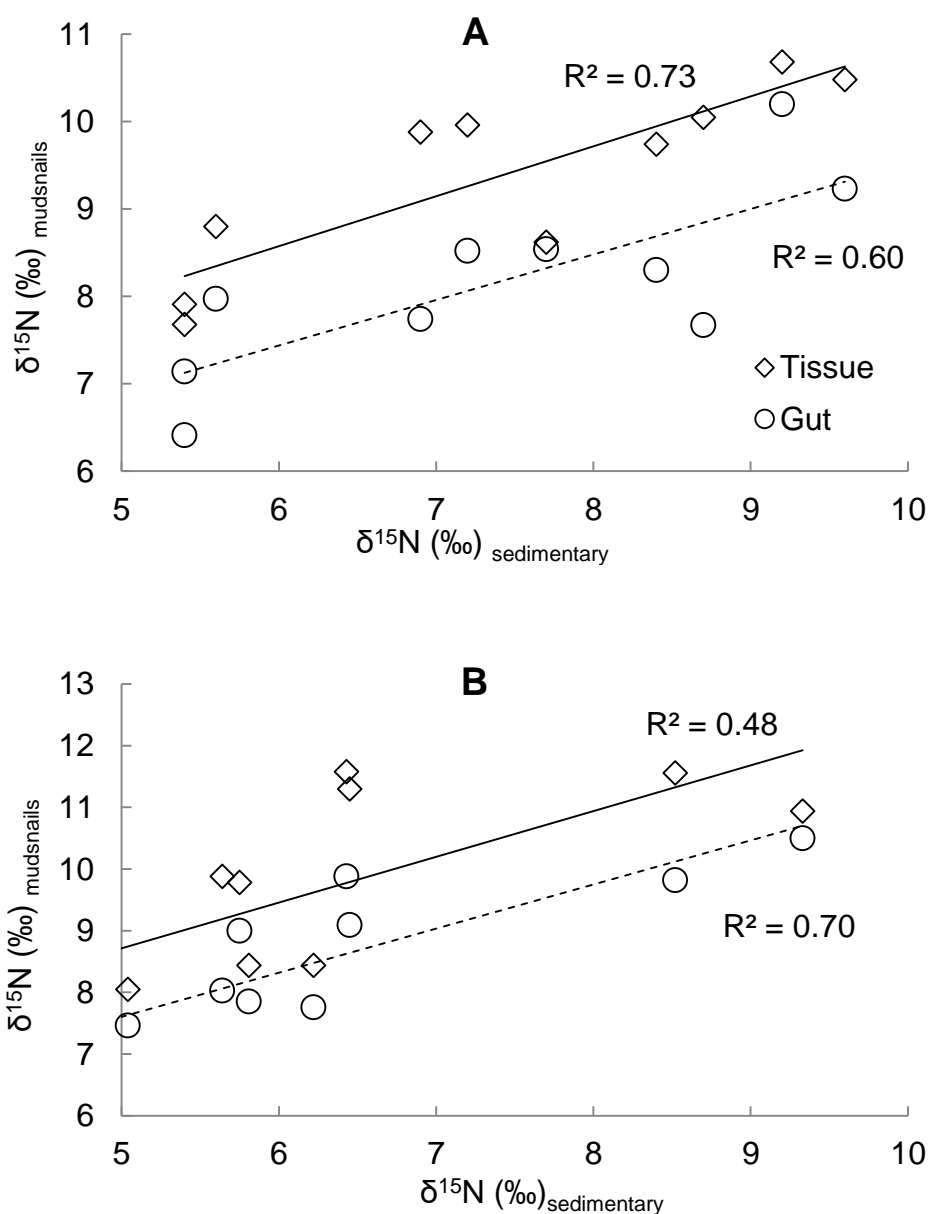


Fig. 4.16. $\delta^{15}\text{N}$ in tissue and gut of mud snails and sediment collected in A. summer and B. winter in Rangataua Estuary. Dashed correlation line: gut

$\delta^{15}\text{N}$ values in mangrove displayed important variation for different season and locations, while $\delta^{13}\text{C}$ remained similar (Table 4.8). $\delta^{15}\text{N}$ values were higher at the West pond in summer (12.5‰) and lower in winter (5‰). It demonstrated that mangrove was very sensitive to seasonal changes in nitrogen origin. At the river, $\delta^{15}\text{N}$ values in winter were greater (7.6‰) than summer (6.6‰).

Table 4.8. Mean (\pm S.E.) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in mangrove in Rangataua Estuary in summer and winter

| mangrove | Summer | | Winter | |
|-----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Site | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ | $\delta^{15}\text{N}(\text{‰})$ | $\delta^{13}\text{C}(\text{‰})$ |
| River | 6.62 ± 0.05 | -26.97 ± 0.44 | 7.6 ± 0.13 | -26.21 ± 0.16 |
| West pond | 12.51 ± 0.45 | -25.38 ± 0.20 | 4.97 ± 0.92 | -26.74 ± 0.21 |

4.4 Discussion

Estuaries are highly dynamic and complex ecosystems due to their hydrological and ecological character further complicated by the presence of numerous human infrastructures potentially affecting the estuarine nutrient budget. The input, exchange, and processing of organic matter and nutrient sources in these systems are similarly complex, but it is this dynamic that we must examine in order to understand ecosystem functioning as a whole, and the connectivity of estuaries with both terrestrial (or riverine) and coastal marine habitats. The purpose of this study was to examine the level of eutrophication (nitrogen and phosphorus) in surface sediment in urban and rural estuaries and subsequent consequences in the biota. While considering estuaries as a whole system in the determination of food web structure, the determination of sources and fates of nutrients has been achieved by separating each estuary into sub-areas.

Eutrophication associated with nutrient inputs of nitrogen and phosphorus from anthropogenic activities can cause severe damage to estuarine environmental health. The finding of nutrient sources is of importance in the development of remediation plans to support environmental managers. Finally, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ associated with total nitrogen, carbon and phosphorus permit elucidation of sources of contamination and their fates in estuarine ecosystems (Zhou *et al.*, 2006; Ye *et al.*, 2014; Mazumder *et al.*). Nitrogen and phosphorus were found in high concentration in urban and agricultural impacted estuaries, with lower level found in the Ohiwa Harbour. Sources of nutrients were mainly related to human

activities as demonstrated by stable isotopes analysis. For example, the use of stable isotope analysis permitted identification of treated wastewater in the sediment and biota (higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values) and allowed determination of the spatial occurrence of this pollutant.

Understanding species interactions in estuarine food webs will help in the detection of human disturbances, the localisation of contaminant sources and the assessment of potential interaction effects among species (Montoya *et al.*, 2006; Hussey *et al.*, 2014). Macroalgae blooms can potentially limit the amounts of nutrients available for other species (Pedersen & Borum, 1997) and can cause direct invertebrate mortality by covering entire shellfish beds (Marsden & Bressington, 2009). The use of stable isotopes in the study of food web dynamics permitted a better understanding of species interactions and nutrient transfers linked with relationships between prey and predators. Mud whelks were a close top predator/grazer in our food web, sharing common food sources as mud snails such as sea lettuce. Sea lettuce blooms were occurring mainly in summer and generated a cascade of consequences from sediment to biota. For example, surface sediment displayed strong seasonal differences in $\delta^{13}\text{C}$ values in the Rangataua estuary with higher values recorded in summer due to an increase of primary production. The higher abundance of sea lettuce in summer was recorded in the tissues and associated with a lower concentration of phosphorus, probably due to the depletion of this element in the water column. However, the lower phosphorus content was not recorded in seagrass tissues suggesting that these two species were not competing for nutrients uptake. Finally, the isotopic ratio of benthic species will constitute a robust baseline for future research.

4.4.1 Surface sediment

Elemental and isotopic analysis in surface sediment has been widely used in the determination of the level of eutrophication and identification of nutrients sources (Savage, 2005; Wang *et al.*, 2015b). Sediment is of fundamental importance as a sink and source for nutrients during adsorption-desorption processes due to sediment-nutrients interaction affected by the flow, grain size and mineralogy, salinity and pH (Abdulgawad, 2010), and will influence the dissolved inorganic budget of nutrients bioavailable (Stumm, 1992). At the present time, the degree of eutrophication affecting the Tauranga Harbour still has to be discovered as well as their respective sources.

In the estuarine ecosystems examined in this study, surface sediment displayed large variation of nutrient concentrations and isotopic ratios across different estuaries and sub-areas. The advantage of sampling sediment was the unlimited spatial availability for collection compared to biological indicators that can have heterogeneous distribution. Furthermore, sediment was not only representative of the present nutrients levels, but can provide an indication of past contamination events. Sediment analyses permit the delineation of potential risks of contaminants. TN and TP content in urban estuaries were higher than levels found in the rural environment with sources generally related to organic content and terrestrial input as well as point sources such as stormwater. This study was in agreement with other findings which identify a positive correlation between nitrogen load and watershed population density (Mouri *et al.*, 2011; McIver *et al.*, 2015). This research illustrated common environmental degradation patterns associated with estuaries that receive water from catchments having similar land uses, such as Waimapu, Rangataua and Waikareao estuaries (Chapter 2). However, the levels of nutrients were different for each estuary demonstrating that the

distribution of nutrients was complex and further research is needed to elucidate the origins of these fluctuations. TN in surface sediments was higher in Waimapu, while Waikareao and Rangataua demonstrated lower and similar concentrations; Rangataua had the highest phosphorus content. The concentration of phosphorus in all estuaries in this study, including rural, were higher than levels found in other estuaries in China, USA, Brazil and Italy (Table 4.3). TN in Waimapu had similar levels to those found in the Daya Bay in China, and TN concentrations in Waikareao and Rangataua were similar to an estuarine system in Italy (Table 4.3). However, Chesapeake Bay, USA is reported with a very eutrophic environment, recording the highest nitrogen content found in sediment. It was clear that eutrophication in Tauranga Harbour, mainly associated with phosphorus input, was not negligible. The identification of pollution sources will permit a better understanding to how to remediate to nutrient loading in the harbour.

A nested study in Rangataua estuary including rivers and streams, stormwater drains and middle harbour indicated that TN and TP were generally present in higher concentrations near freshwater inputs. However, nitrogen and phosphorus had different sources as shown by stable isotope analysis. In Waimapu, TN was higher in the wetland and the river outlet with a decreasing gradient with increased distance from the river. This was confirmed by an increase of TN with lower $\delta^{13}\text{C}$. This was similar to the result of trace element concentration patterns demonstrated in Chapter 2, with the wetland acting as a natural scrubber of toxic trace elements. It illustrated the fundamental role, or property, of wetlands in trapping nutrients. In Waikareao, higher concentrations of TN were found near the stormwater drain and the outlet of the stream. Rangataua displayed a similar pattern as Waikareao, with higher TN found at the river and drain which indicated that the WWTP was not the main contributor of nitrogen in

this estuary. Tuapiro Estuary had similar concentration of TN as the outlet of Waikareao Estuary, demonstrating that agricultural activities directly influenced the TN budget of the entire estuary. Ohiwa Harbour was the least impacted estuary with fewer nutrients found in surface sediments.

Analysis of $\delta^{13}\text{C}$ values permitted assessment of the sources of nitrogen which increased with lower $\delta^{13}\text{C}$ values and were correlated with terrestrial input. TP concentrations at the study locations were dissimilar to TN, which were associated with an increase of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Higher concentration of TP was correlated with a freshwater source for Waimapu and Rangataua as well as point sources of pollutions. These point sources included the WWTP and the drain for Rangataua; and industrial activities localised around the port for Waikareao, possibly due to the production of superphosphate fertiliser in the area. Higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values near WWTP indicated seepage of treated wastewater in Rangataua estuary, where signals were typical of N fractionation by bacterial processing on N species during wastewater treatment (Heaton, 1986; Savage, 2005; Hartland, *et al.*, 2011). However, enriched $\delta^{15}\text{N}$ signatures were correlated with an increase of TP. In Rangataua estuary, nitrogen content in sediment was not associated with the contamination of treated wastewater, while phosphorus showed an opposite trend most likely due to treated wastewater contamination.

The transect from the WWTP towards the outer part of the estuary allowed examination of the extent of influence of leaked treated wastewater as far as 1 km from the treatment plant. This result was greater than the estimation based on visual observation of ~20m made by the Tauranga City Council. The sedimentary enrichment of $\delta^{15}\text{N}$ was more marked in winter than summer suggesting a greater influence of the WWTP in winter, possibly due to increased assimilation and

removal of sewage-enriched N during the more productive summer months. However, we found that higher $\delta^{15}\text{N}$ values were more associated with phosphorus than nitrogen, and probably other trace metals as shown in Chapter 2. In Rangataua, the dual use of stable isotope assessments permitted examination of two additional sources of nutrients with distinctive stable isotope ratios. They were localised at the drain and another unknown source localised 1200 m away from the pond, near the motorway. Thus stable isotope analysis appeared to be a good technique in the localisation of unknown pollution sources. In Rangataua, the seasonal effects of TN in surface sediment were compared to TP. Changes of TP between summer and winter was probably due to the removal of TP by the increase of productivity of phytoplankton and macroalgae during the summer period. Examination of isotopic ratios on their own did not allow full assessment of the degree of contamination, and needed to be correlated with elemental data such as TN and TP.

In this section, we clearly demonstrated that eutrophication was due to numerous sources of pollution affecting the Tauranga Harbour. Different concentrations of nitrogen and phosphorus associated with contamination could be found for different estuaries. The N and P sources were not always associated as shown by different correlations with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. However, concentrations in surface sediment alone provide only limited information on how P and N affect the magnitude of primary production or export. It is of equal importance to determine seasonal fluctuation of nutrients in the tissue of algae and invertebrates as documented in the next sections. In the next section (4.4.2) for the first time in Tauranga Harbour, we will present the analysis of water samples in Rangataua estuary to evaluate the potential use of $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ to complement sedimentary analysis in the detection of treated wastewater.

4.4.2 Surface water

The analysis of $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ in water has only been recently applied in the study of wastewater (Gammons *et al.*, 2011; Minet, *et al.*, 2012) and never applied in the Bay of Plenty. In estuarine environments, the pollution signal could be rapidly lost due to fast exchange of water during tidal movement. However, in surface sediments and benthos, such signals are likely to be integrated through time. In this section, we investigate if this novel technique could be used for the assessment of wastewater treatment plant contamination in Rangataua estuary. $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ in surface water displayed seasonal and spatial variation with clear segregation between samples collected within the oxidation ponds and others collected directly from the Rangataua estuary. $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values in pond 2 displayed more seasonal variation than pond 3 with higher $\delta^{15}\text{N-NO}_3$ values. $\delta^{15}\text{N-NO}_3$ values in winter were higher than summer in Pond 2 and slightly lower for pond 3. These results indicated variability in the quality of the wastewater treatment discharged into Ponds which leaked into the estuary; it may be due to lower efficiency in the treatment of wastewater in winter. The mean daily inflow of raw sewage in September (19758 m^3) was higher than March (16863 m^3) potentially affecting the quality of the treatment. This observation was supported by the smaller seasonal variation of $\delta^{18}\text{O-NO}_3$ observed in summer water samples and higher $\delta^{15}\text{N}$ values of sediment and tissue and gut of mud snails near the ponds in winter.

Water samples collected in the Rangataua Estuary displayed seasonal and spatial variation. In summer and winter, lower $\delta^{15}\text{N-NO}_3$ values were found near the drain and stream and higher values in the Centre pond. The large variation in $\delta^{18}\text{O-NO}_3$ in the Centre pond and middle harbour site, could be an indicator of a mixture of sources ranging from fertiliser and rain, sewage and atmospheric

deposition (Kendall & Caldwell, 1998). Seasonal variation in $\delta^{18}\text{O}\text{-NO}_3$ values were small for sites close to point sources such as the drain and the stream with isotopic signatures close to agricultural fertiliser (Kendall & Caldwell, 1998). These results confirmed that $\delta^{18}\text{O}\text{-NO}_3$ values were a valuable indicator to use when discriminating source of contamination, especially in dual with $\delta^{15}\text{N}\text{-NO}_3$. Enriched isotopic signatures were likely caused by denitrification processes due to isotopic fractionation. This is because microorganisms preferentially use the lighter isotope (^{14}N and ^{16}O), leaving the residual NO_3^- enriched in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. $\delta^{15}\text{N}\text{-NO}_3$ in surface water displayed similar fluctuations than surface sediments mainly associated to proximity of contaminants sources such as drains and treated waste water.

From this research, it appeared that the analysis of surface water confirmed sedimentary analysis in the identification of pollution sources such as stormwater drains and the presence of treated wastewater. However, scarce water measurements were less clear than surface sediment analyses and more costly. The isotopic analyses of nitrate in water appeared to be a great tool in less 'dynamic' waters such as wastewater treatment ponds, to compare the efficiency of a treatment plant at different time of the year.

4.4.3 Autotrophs: mangrove, seagrass and sea lettuce

High concentrations of nitrogen and phosphorus in surface sediment have been previously found (Table 4.3) and will potentially affect primary production in the Tauranga harbour. From visual inspection, the frequency of sea lettuce blooms was higher in summer and there is a need to know if the growth of sea lettuce is limited by either nitrogen or phosphorus. Furthermore, sea lettuce is an opportunistic species potentially competing for nutrient uptake with other species

such as seagrass, as shown by the fast rate of *Ulva* growth and the consumption of large amounts of nutrients (Viaroli *et al.*, 2005; Teichberg *et al.*, 2010).

Finally, we examine the advantage to use primary producers as a complementary indicator to sediment in the detection of pollution events. Mangrove, sea lettuce and seagrass had similar C:N:P ratios as primary producers relying on photosynthesis mechanisms. TP found in algal tissues reflected the concentration of nutrients available in the water column (Fong *et al.*, 1994). Thus larger variation of P in sea lettuce tissues at different locations can be attributed to more variation in the water column. TN and TP concentrations were higher in winter than summer which highlighted the role of sea lettuce to consume large amount of nutrients. Lower TN and TP concentrations in summer were correlated with an increase of primary production due to sea lettuce blooms. High proliferations of sea lettuce cause dilution of nutrients in the autotroph species when the metabolic demand exceeds assimilation of nutrients from the water. The determination of nutrient limitation for many primary producers is often based on the examination of nutrient ratios (Fourqurean *et al.*, 1992; Larned, 1998; Kähler & Koeve, 2001; Davidson, *et al.*, 2012; Zhang, *et al.*, 2015). Seasonal comparison of C:N:P ratios in seagrass, sea lettuce and mangrove permitted examination of nutrient requirements and possible competition in the uptake of nutrients between species. Seawater typically has a relatively fixed Redfield ratio of 106:16:1. Deviations from these ratios provided preliminary evidence for specific processes controlling how much of an element is present but, it can be dangerous to use elemental ratios as the only evidence for nutrient limitation. When $N:P < 16$, the system may be nitrogen limited (excess phosphorus), $N:P > 16$ system may be phosphorus limited (excess nitrogen) (Neill, 2005). The Redfield ratio may be reflective of unicellular organisms; however because of the structural components

associated with macrophytes the classic oceanic Redfield ratio (C:N:P = 106:16:1) is not appropriate (Atkinson & Smith, 1983). Elemental ratios can provide a general rule of thumb for nutrient limitation. Literature reviews indicate that the median leaf seagrass C:N:P is about 400:20:1 with considerable variability. Additionally, other nutrients can play a role in controlling seagrass production, for example Herman *et al.* (1996) present a study where they suggest that decreases in dissolved silica may have been a factor in seagrass decline. It is more likely that sea lettuce will be the species having the most effect on nutrients limitation due to its fast growing characteristics in estuarine system. Total nitrogen content measured in sea lettuce tissues were high compared to other studies and was significant for areas characterised by high nitrogen loading (Barr, *et al.*, 2013). In this study, sea lettuce C:N ratio did not show any strong variation between summer and winter while a clear increase of C:P in summer was found. It is therefore evident that P is the main limiting nutrient for primary growth in summer. This was confirmed by TP concentration in surface sediment which was lower in summer in Rangataua. Due to the abnormal growth of sea lettuce in summer, the quantity of P taken up by this macroalgae was high and potentially limited the availability for other species such as seagrass. However, the seasonal variation in C:N:P ratio in seagrass was small compared to other systems where P was limiting the growth of seagrass (Fourqurean, *et al.*, 1992). The P limitation for the growth of sea lettuce was confirmed by comparing TN and TP in sea lettuce with critical concentrations calculated from other studies (Lyngby *et al.*, 1999; Villares & Carballeira, 2003). TN in summer and winter was above the critical concentration of 2.4% which indicated that there was enough nitrogen in the system to support the growth of sea lettuce. However, TP in sea lettuce was below the critical concentration in summer established as 0.08% which indicated

that the concentration of P in the water column was not high enough to support the exponential growth of sea lettuce.

Stable isotope analysis demonstrated different pathways in elemental uptake as well as providing further information on pollutant sources. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were not the same between species due to differences in the uptake and assimilation of nutrients as well as their locations and ecological interactions. Mangrove had depleted $\delta^{13}\text{C}$ compared to other species due to C_3 photosynthetic biochemistry (Smith & Epstein, 1971; Farquhar *et al.*, 1982). Mangrove had a wide range of $\delta^{15}\text{N}$ from -2‰ to 12‰ characteristic of different sources. Higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios were found on the edge of wastewater ponds confirming the release of treated wastewater. In Tuapiro Estuary, low $\delta^{15}\text{N}$ values in mangrove were characteristic of the value found in fertiliser (-2‰) thus identifying the main sources of nutrients in this estuary which were heavily impacted by agricultural practises in adjacent catchments. The large variation of $\delta^{15}\text{N}$ found in mangrove make them very sensitive to changes in nutrient sources due to their location on the edge of estuaries.

$\delta^{13}\text{C}$ values in seagrass were lower compared to sea lettuce and showed a wider range of extreme values. Lower $\delta^{13}\text{C}$ ratio was due to enzymatic and physical processes such as the presence of root system and the capacity to absorb CO_2 from the atmosphere during emersion in low tide (Cooper & DeNiro, 1989; Leuschner & Rees, 1993). Sea grass was found at close proximity to oceanic inputs as well as the edge of mangrove forest. It could be another parameter explaining the large variation of $\delta^{13}\text{C}$ values, with seagrass recycling the carbon available in their closed environment. The mineralisation of mangrove litter will result in the formation of CO_2 with a $\delta^{13}\text{C}$ values close to the $\delta^{13}\text{C}$ low values of

living mangrove biomass. In contrast, sea lettuce displayed higher values due to C_4 carbon uptake pathways deriving inorganic carbon from dissolved inorganic carbon (HCO_3).

4.4.4 Invertebrates: cockles , oysters, mud snails and mud whelks

Stable isotopes analysed in muscle and gut tissues were a useful tool to distinguish different food sources between estuarine invertebrates. Invertebrates displayed variation in their $\delta^{13}C$ and $\delta^{15}N$ ratio due to different feeding habit as well as their proximity to the influence of pollution sources. While cockle and oyster are both water filter feeders, $\delta^{13}C$ and $\delta^{15}N$ in oysters were lower than cockle with higher variation between gut and tissues. $\delta^{13}C$ and $\delta^{15}N$ values found in cockle were similar to a previous study (Hailes, 2006) and were mainly related to the absorption of microphytobenthos which shared similar $\delta^{13}C$ to cockle (Hailes, 2006). Lower $\delta^{13}C$ ratios in oysters in the present study were very similar to isotopic ratio found in phytoplankton (Hailes, 2006), which seems to constitute most of their diet. The differences in nutrient sources could be attributed to the differences of locations between cockles and oysters, the former found in surface sediments and potentially more sensitive to resuspension of microphytobenthos than oyster, attached to rocks in the upper water column and less affected by micophytobenthos resuspension. The source of food is of primary importance in the investigation of the origin of food contamination and ecotoxicology. $\delta^{15}N$ ratio in mud snails were greater compared to cockle and oyster, but similar in value to sea lettuce. Mud snails are deposit feeders grazing on the surface of sediment and the isotopic ratio indicated that they mostly fed on decomposed matter from different origins (e.g. sea lettuce and seagrass). This line of evidence was reinforced by comparing isotopic ratio in mud snails with sediment, which showed a positive relationship. $\delta^{15}N$ in tissues and gut increased with sedimentary $\delta^{15}N$. Isotopic

fractionation between tissues and gut of mud snails were higher for the stream and Center pond with reported values of $\sim 2.5\text{‰}$ in summer and $\sim 2\text{‰}$ in winter for carbon and nitrogen. A trophic shift for carbon has been recorded under special conditions, for example for longer lived animals after final weight has been reached. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in mud whelks were more enriched than mud snails due to the higher trophic position as carnivores and thus feeding on a wide range of species including fish carcasses and other carrion. This result contradicted most other published research findings, which routinely reported a trophic enrichment of $\sim 2.5\text{‰}$ for nitrogen and $\sim 0\text{--}1\text{‰}$ for carbon (DeNiro & Epstein, 1978, 1981; Peterson & Fry, 1987), due to differential digestion or fractionation during assimilation and metabolic processes.

While the feeding habit was different between invertebrate species as shown by isotopic analysis, the C:N:P ratios were similar between cockle and oyster, and between mud snails and mud whelks. Thus despite major differences in the size, diet, and taxonomic affiliation, patterns of biomass for composition for N and P were very similar. Cockle and oyster as well as mud snails and mud whelks seem to share similar nutrient requirements. Mud snails were an excellent indicator for tracking of contaminants especially of treated wastewater. Mud snails reflected sources of pollution not influenced by seasonal variation, i.e. characteristic of a constant input, such as the Drain in Rangataua Bay. This species was more tolerant of pollution than seagrass and sea lettuce, and showed clear differences with higher $\delta^{15}\text{N}$ values for sites at proximity of WWTP and further away. Mud snails appeared to be a great indicator in the determination of nitrogen sources.

4.5 Conclusion

Elemental content and isotopic ratios of surface sediment, mangrove, seagrass, sea lettuce and mud snails were highly variable across estuaries demonstrating the importance of diverse sources of pollutants. This study provided the first baseline investigation on elemental stoichiometry and isotopic ratios for Bay of Plenty. The analyses demonstrated the use of stable isotopes and C:N:P ratios for the detection of anthropocentric inputs, species interactions related to their diet, as well as nutrient requirements and limitations due to blooms of macroalgae.

From this research, it was clear that the entire Tauranga Harbour system was influenced by loading of nutrients having multiple cascade effects on the ecosystem. Furthermore, urban estuaries had higher TN and TP content than rural estuaries and clear differences were observed between rural areas heavily impacted by agriculture (Tuapiro); than less impacted environment (Ohiwa). Stable isotope analysis permitted the detection of human and industrial waste from WWTP. However when assessing the influences of these sources, it appeared that elemental content such as nitrogen and phosphorus, and probably other contaminants such as heavy metals, need to be included in the design of the study to quantify the effect of such contamination. In fact, while higher $\delta^{15}\text{N}$ was found closer to the WWTP pound, it was correlated with an increase of phosphorus rather than nitrogen. While the isotopic analysis of sediment was useful in the determination of WWTP and its spatial extend, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in biota allowed detection/ confirmation of the impacts of WWTP, stormwater drains and the high use of agrichemicals such as fertilisers .

Here we presented the first investigation in the Tauranga Harbour to examine nutrient dynamics and limitation (nitrogen or phosphorus) due to blooms of sea lettuce. The seasonal analysis of C:N:P ratios in tissues of mangrove, seagrass, and sea lettuce permitted an increase in our understanding of nutrient requirements and limitations. This study demonstrated that opportunistic algae (sea lettuce) with simple morphology and fast maximum growth rates, suffered more from nutrient limitation than species with a more conservative growth strategy (seagrass). It was shown by comparing C:N and N:P ratios between mangrove, seagrass and sea lettuce, that more variation was found in sea lettuce. Nitrogen was not limiting the growth of autotrophs, reinforcing the likely high concentration derived mainly from river input in Tauranga Harbour waters. Phosphorus was the main limiting nutrient for algal growth as shown by seasonal variation and tissue concentration under the critical growth phase for sea lettuce. A reduction in phosphorus from more sustainable management of its use, together with better management of nitrogen on the land, is the principal remediation measure to reduce blooms of sea lettuce.

The reconstitution of species diet was examined by the analysis of muscle and gut content, which permitted food source characterisation between species, including those sharing similar feeding strategies such as filter feeders. By the dual use of nitrogen and carbon isotope analyses, we were able to discriminate food sources between cockles which were mainly accumulating microphytobenthos, and oysters that were feeding mostly on phytoplankton. This is of fundamental importance when assessing the potential sources of contamination as diet can be one of the main pathways responsible for the accumulation of contaminants.

While the estimation of pollution sources was possible by stable isotope analysis and the measurement of TN and TP content, it appears that further research is needed in the understanding of nutrient limitations associated with primary production. While the quantity of N and P removal by phytoplankton blooms has been measured as negligible compared to macroalgae (Sfriso *et al.*, 1989), phytoplankton still needs to be assessed in parallel to water measurement. Possible cross competition between phytoplankton, sea lettuce and seagrass in nutrients uptake could be addressed in the understanding of nutrient dynamics.

5 Chapter Five

Microhardness and elemental composition of cockle shell from an environmental pollution gradient: EcoHardness as a new environmental indicator

5.1 Introduction

Trace elements are fundamental for the existence of life on earth. The chemical composition of a local environment, which can be altered by anthropogenic inputs, influences the accumulation of trace elements within organisms. This can in turn affect the health of organisms and their long-term physiological functioning. Trace metals are often discharged from urban environments and agriculturally intensified catchments, and now are considered as the third most important contaminants in estuaries after organic matter and oil (Luoma, *et al.*, 2008; Legorburu *et al.*, 2013; Xu *et al.*, 2014b). Trace metals are usually present in low concentrations in the natural environment; however, their local concentrations can be greatly increased by the presence of natural or anthropocentric sources (Alsina *et al.*, 2014; Yang, *et al.*, 2015b). In New Zealand, natural events releasing trace metals are often associated with emissions from volcanic vents and geothermal springs (Hedenquist & Gulson, 1992; Wardell, *et al.*, 2008). Human activities that increase trace metal concentrations include industrial activities such as the mining of minerals including the extraction of fossil fuels (Tornero, *et al.*, 2014) and the intensive use of fertilisers and pesticides by the agricultural sector (Gimeno-García, *et al.*, 1996; Xu *et al.*, 2014a).

Estuaries are at the interface between the terrestrial and oceanic environment and are important habitat for numerous species including birds, fishes and plants. Due to their location and physical configuration, the water exchange flow in estuaries is usually reduced and mainly influenced by tidal movements; thus estuaries are a sink for many pollutants, including trace metals (Buggy & Tobin, 2008). Increased concentrations of trace metals in estuarine ecosystems has been demonstrated to be toxic for estuarine species as well as to human consumers (Ip *et al.*, 2005; Sivaperumal *et al.*, 2007; Serafim *et al.*, 2013). The distribution and accumulation of trace metals have thus been monitored in the last few decades to remedy adverse consequences of pollution in the ecosystem. The fate and effect of trace metals has been internationally researched and are now well established; however, the ability to examine metal properties at a microscopic level through advanced technology (e.g. scanning electron microscopy with X-ray microanalysis) permitted elaboration of new ways to investigate metal/physiological processes (Nuñez *et al.*, 2012; Ahmad *et al.*, 2013; Zuykov, *et al.*, 2013).

The common cockle, *Austrovenus stutchburyi*, is a marine bivalve mollusc in the Veneridae family. Large cockle (shells > 35 mm) were very abundant 50 years ago, but are now rare in most places within the Tauranga Harbour (Taiapa *et al.*, 2014). Mass mortalities and abnormal growth morphologies are of particular concern for the species given their ecological role as well as the fact that they are harvested by tangata whenua. Ecologically, cockle beds are of considerable importance in maintaining a balanced biodiversity in terms of estuarine trophic webs. For example, a reduction in quahog density directly modified lower trophic levels such as phytoplankton and zooplankton biomass, likely leading to the appearance of blooms and potentially having additional consequences throughout

the ecosystem (Lonsdale *et al.*, 2007). These animals provide a food source for higher trophic levels, such as shorebirds, and fishes (Norris *et al.*, 1998). In Caraguatatuba Bay in Brazil, a study found that the triangle clam (*Tivela mactroides*) was consumed by 21 different species (Turra *et al.*). Laboratory studies demonstrated that shore crabs can consume up to 40 cockles per individual.day⁻¹ with preferences for smaller prey (Sanchez-Salazar *et al.*, 1987).

The morphology of cockles in NZ comprises laterally compressed soft bodies enclosed by two distinctly convex hinged valves. Cockles cannot swim but bury themselves in the sand at a depth of approximately 2 cm, seeking shelter and protection from many predators including various species of starfish and snapper (*Pagrus auratus*). The molluscan shell is formed, repaired and maintained by the mantle. Any injuries to or abnormal conditions of the mantle are usually reflected in the shape and form and even colour of the shell (Wagge, 1951). Structural integrity of the shell is important for survival. Differences or changes in the chemical composition of the ambient micro-environment, including water, sediment, phytoplankton and other fine particles, will directly affect mantle chemical composition. The extra-pallial fluid, which is enclosed between the mantle and the inner shell surface, is the medium in which the organic matrix and the crystalline components of shell are formed. Calcium carbonate deposition is a function of the calcium ion concentration in the extrapallial fluid, which in turn is dependent on the amount entering the body. A marked reduction of the calcium concentration of seawater may reduce or even prevent calcification. Cockle shells are comprised of three calcified layers (Fig. 5.1): an outer layer with a crossed-lamellar microstructure, a middle layer of homogenous crystals and an inner layer with complex crossed lamellar or homogenous structure (Meenakshi *et al.*, 1974).

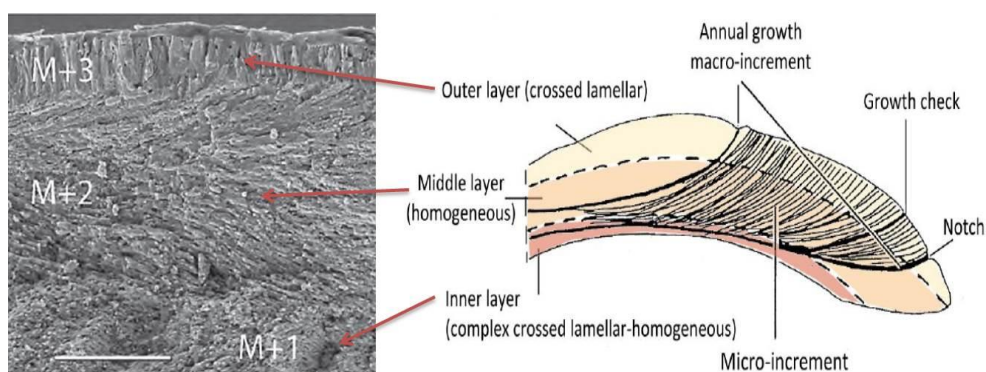


Fig 5.1 Schematic cross-section and microstructure of clams showing zones of different shell microstructure (crossed lamellar, homogeneous and complex crossed lamellar). Figure modified from McKinnon, 1996 and Bieler et al, 2014.

Bivalves can also incorporate metals within their shell matrix during the process of biomineralisation. This process occurs in two distinct ways. Either metals may be included within the organic matrix that binds the various bivalve shell layers, or metals with similar configuration to calcium may directly occupy a place within the shell (Lingard *et al.*, 1992). Trace metals have been measured in bivalve shells to monitor the environmental and chemical state of estuaries, sources of metal pollution and changes through time (Gillikin *et al.*, 2005; Pearce & Mann, 2006; Junchaoenwongsa *et al.*, 2011; Ahmad, *et al.*, 2013). Past studies have shown that the content of metals recorded in shells can often be higher than in soft tissues, and metals in shells have longer biological half-lives. For example, a study comparing elemental compositions between soft tissues and shells in control (unpolluted) and contaminated sites showed that Mn was found in higher concentration in the shell than in tissues, and an opposite trend was found for Fe, Zn, Cu, Cd and Co. They concluded that shell could be a good indicator for Mn contamination (Cravo *et al.*, 2004). However, the data available in numerous papers are based on the concentration of metals on the external shell surface, while concentrations on the internal shell surfaces were considered lower and often negligible (Thorn *et al.*, 1995; Zuykov *et al.*, 2011; Gillikin & Dehairs,

2013). The phenomenon of trace metal accumulation in shell therefore can be used as an indicator. Pourang *et al.*, (2014) investigated the distribution of trace elements in the soft tissues and in the shell layers of the pearl oyster, *Pinctada radiata*. They found the oyster as a suitable proxy for measuring temporal and spatial variation of trace elements. Historical lead content has been investigated in the quahog clam *Mercenaria mercenaria* in North California (Gillikin, *et al.*, 2005). They were able to measure variation of lead from 1949-2002 and clearly exhibited a late 1970s increased of Pb from leaded petrol use.

The hardness of a material characterises its resistance to penetration. It also characterises the intensity of the atomic bonds, structure and crystallisation. Microhardness has been widely used in engineering and material science, for example, when looking at the consequences of different elemental compositions and micro-structures under different thermal parameters (Dias *et al.*, 2014; Tang *et al.*, 2014; Zhao *et al.*, 2015). Metals have variable hardnesses ranging from very soft (Ca) to very hard (W, U). Microhardness has been measured and associated with different structures of the bivalve shell (Yang *et al.* 2011), who found that the microhardness in the inner part of the shell, associated with cross lamellar structure was the hardest. Microhardness in the middle layer was lower than the inner layer, and the lowest values were found in the outer layer (Zhu *et al.*, 2006; Yang *et al.*, 2011). They found that most variations in microhardness occurred within the inner layer and was associated with microstructure. Similar trends of microhardness were found in other studies using two different species of brachiopods (Pérez-Huerta *et al.*, 2007), with the calcite semi-nacre (inner shell) providing harder and stiffer structure. Furthermore, they were able to relate microhardness value to the mode of life such as specific adaptation to different ecological environment. However, the use of microhardness to fingerprint

different environments characterised by specific chemical composition and pollution stress has never been investigated in environmental science.

This study presents the first attempt to measure microhardness in the ventral outer edge of cockle shells endemic to New Zealand estuaries for the purpose of investigating potential influence of metal accumulation in cockles from their environment. Here, we use cockle shells to assess the effects of changing in calcite concentration in different environments. The aim is to determine the feasibility of using microhardness as a new environmental parameter to fingerprint elemental variation between estuaries. The specific objective is to measure and compare microhardness with 16 trace elements found in sediment, tissues and shells of cockle. Furthermore, this study aims to increase our understanding of the chemical fates of metabolised and adsorbed trace metals between different anatomies of the cockle (outer and inner parts of the shell and tissues).

In this chapter, we hypothesise that trace metals and microhardness measured in cockle shell would be different between a control site and other locations affected by trace metal contamination. Microhardness would be lower in the control site due to higher calcium content in the shell and inversely increase in the contaminated environments associated with a decrease in calcium content in the cockle shell. This assumption is based on the low microhardness value of the calcium element compared to other trace metals.

5.2 Method

5.2.1 Sample Location

Austrovenus stutchburyi was collected from three different sub-estuaries located in Tauranga Harbour (Fig. 5.2). These urban (Bureta and Rangataua) and rural (Matakana Island) environments are characterised by different levels of pollution stress. The most degraded estuary is located in Rangataua and is affected by point sources of chemicals such as wastewater treatment ponds and stormwater drains as well as non-point source discharges of pollution such as the Waitao River (chapter 2,3 and 4). The Waitao River is one of the most degraded rivers in the Bay of Plenty due to intensive farming activities linked with the intensive use of fertilisers and pesticides in its source catchments. The second area, known as Bureta, is located on the edge of the urban area, where burgeoning industries are present. Due to its location with more direct contact with the main harbour channel, Bureta is prone of more oceanic input than Rangataua. The last area is located on a sand bank next to Matakana Island (control). It is positioned further away from streams, rivers and human activities than the previous sites. Thay (2012) demonstrated that residence times varied across the Tauranga Harbour with increasing residence times in the upper reaches of the estuary and also in sub-estuaries with constructed mouths (6 to 8 days) while the harbour was well flushed with residence times between 2 to 4 days near the harbour mouth.

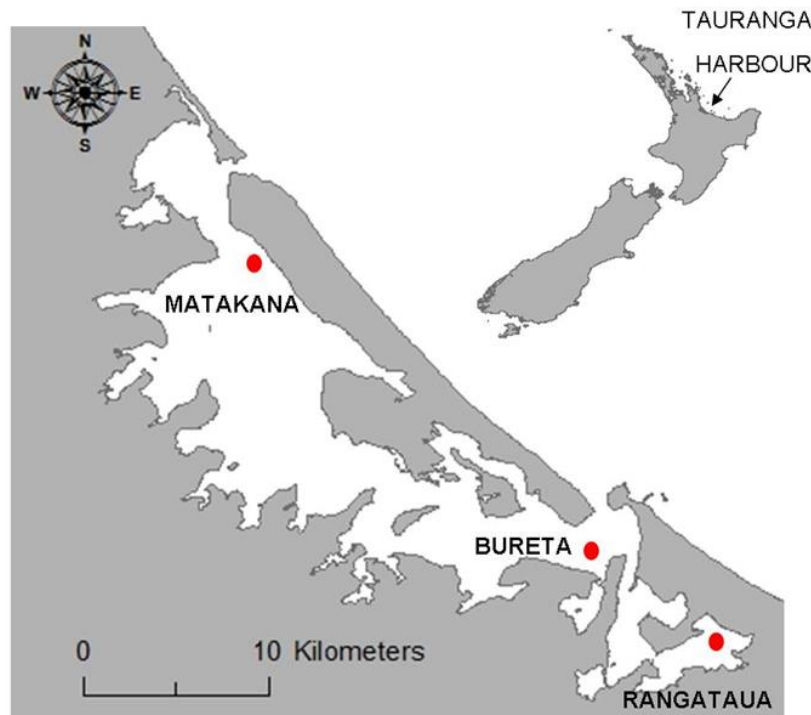


Fig. 5.1. Sample location of collected cockles in Tauranga Harbour.

5.2.2 Cockle distribution

Cockle distribution was assessed in January 2014 by sampling 13 sediment cores at each site (130 mm diameter core extending 150 mm into the sediment) randomly assigned to the sand bed of Rangataua, Bureta and Matakana sites. Cockles were counted and measured on site as well as observed for any visual characteristics including differences in the colour of shells. A total of 20 cockles were collected by hand at each site for further analysis including X-ray fluorescence (XRF), microhardness and scanning electron microscope (SEM) coupled with an energy dispersive spectroscopy (EDS). During the sample collection, precautions (standardisation) were taken by collecting bivalves of same size to avoid metal uptake variation due to different life stages. For example, variation of Pb concentration across sections of shells have been identified, with higher concentration close to the umbo, suggesting that incorporation of Pb into the shell may depend on age (Purchase & Fergusson, 1986).

5.2.3 Analytical details

Elemental Analysis

A total of six cockles per site were carefully dissected to separate the tissue and the shell. Shell and tissue were separately washed with distilled water and dried at 30°C for 12 hours in an oven. The tissues and the hard part of cockles were analysed using X-ray fluorescence (XRF), Spectro XEPOS, which is capable of detecting the elements from Na to U from the periodic system. Each shell was analysed using XRF for both sides (inner and outer side of the shell).

At each site, three replicate of surface sediments were collected with a 20 mm diameter syringe core extending 30 mm deep into the sediment. Six subsamples of surface sediment per site were pooled to create a composite sample. Samples were dried in oven at 30°C for 24 hours and sieved at 64 µm in the laboratory. Fine sediments (< 64 µm) were analysed for elemental analysis by XRF.

Microhardness and Microscopy

Samples of cockle shells collected from the three sites were analysed for microhardness and elemental content at a microscopic scale. Samples of cockle shells were embedded in an epoxy resin for grinding and polishing purposes. The resin-mounted samples were placed overnight in a vacuum chamber (Cast N'Vac 1000, Buehler), as shown in Fig. 5.3a.

Shell sections of the mounted sample (Fig. 5.3b) were then carefully ground by an automatic grinder/polisher (Beta, Buehler, Fig. 5.5a) using three sequential grit sand papers (180, 240 and 320) to remove epoxy resin from the cut surfaces and to reveal the ventral outer edge.

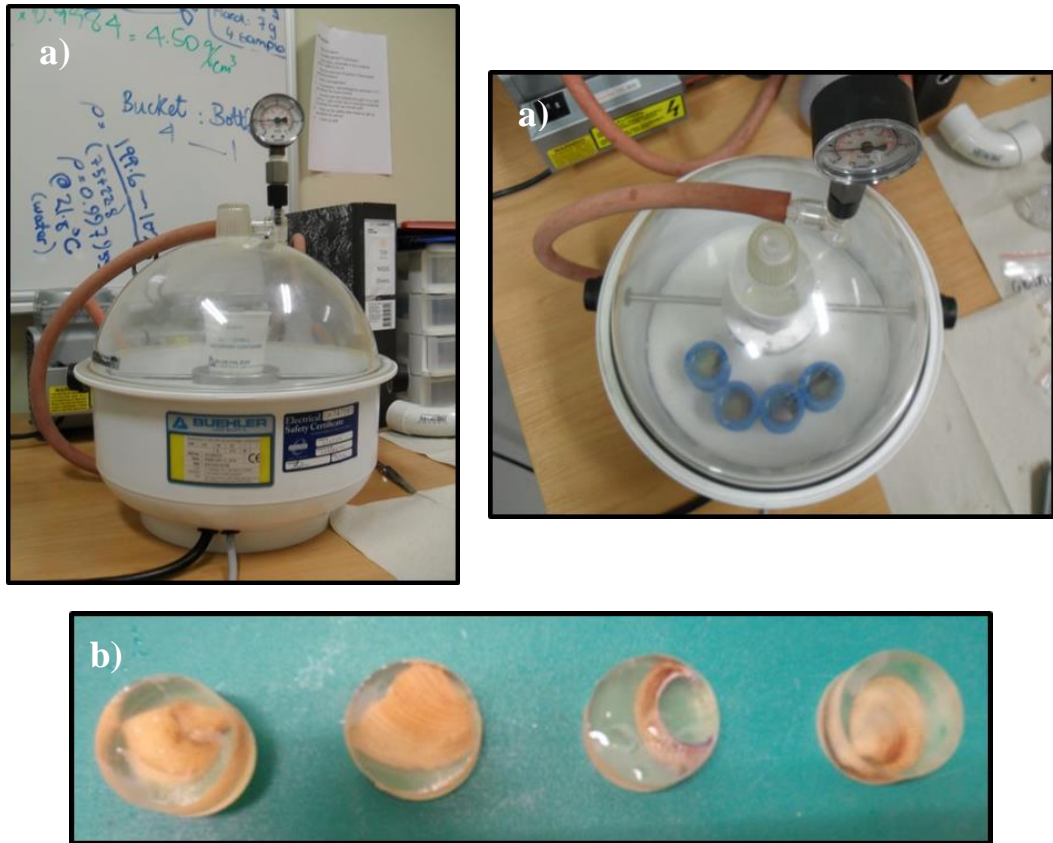
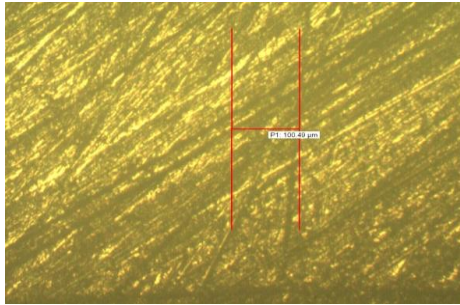


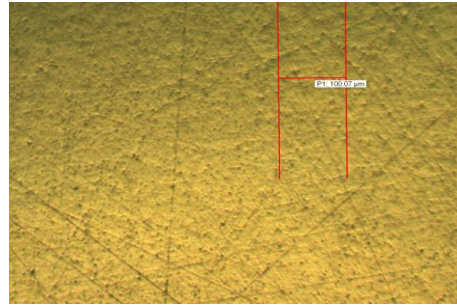
Fig. 5.2. Vacuum chamber (a) of the resin mounted shell sample (b) prepared at the Titanium Industry Development Association.

Precautions to avoid grinding through the shell were taken so only the analysis of the external ventral margin of the shell was possible. Each grit paper was used for 3-4 minutes at a speed of 250 rpm. In the last step of grinding, a finer grit paper (600/1200) was used for 2 minutes to remove scratches. The ground surfaces of the cockle shells were polished using 9 μm diamond suspension (MetaDi Polycrystalline diamond suspension) for 5 minutes at a speed of 200rpm. In the final step, silica polish (Colloidal Silica Polishing Suspension) was used for 2 minutes at a speed of 150 rpm. The same force (25N) was kept constant throughout grinding and polishing steps. An optical microscope connected to a camera was used to examine the microstructure after each step of grinding and polishing (Fig. 5.4). The cross section of the cockle shell, as shown in Fig. 5.2b, was then removed, rinsed in tap water and dried.

AFTER GRINDING



AFTER 1ST POLISHING
9 μm



AFTER 2ND POLISHING
1 μm

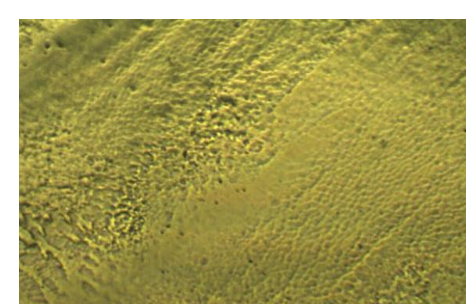
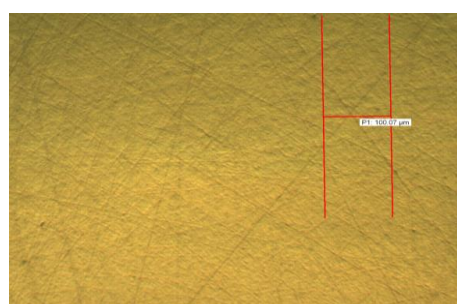
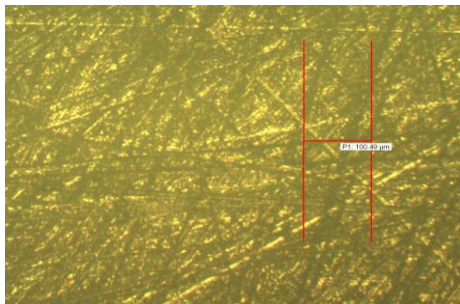
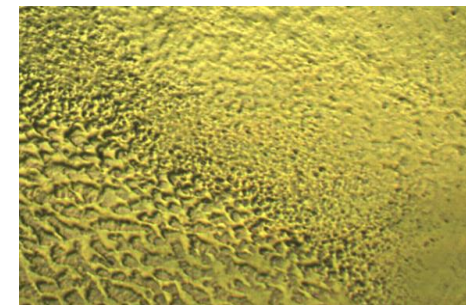


Fig. 5.3. Microscopic imagery observation of cross section of cockle shells after grinding and polishing

A Vickers microhardness tester (Buehler, Micromet 5104) was used to determine the microhardness of resin mounted and polished samples of cockle shells (Fig 5.5b). Ten indents within the inner and the outer part of each cockle shell were examined with a load of 25g and loading duration of 10s. We used Energy dispersive X-ray spectroscopy (EDS) for the determination of elemental concentration at a microscopic level. EDS was examined using Zeiss EVO MA25 scanning electron microscope (SEM) equipped with an energy dispersive X-ray spectrum analyser (Oxford Instruments- Fig 5.5c-d). Two different areas of each cockle shell were analysed at a constant magnification of 500x which each of them was divided into three sub-areas comprising two replicates: the inner, middle and outer parts of the shell to look at variation of trace elements for different areas within the cockle shell.

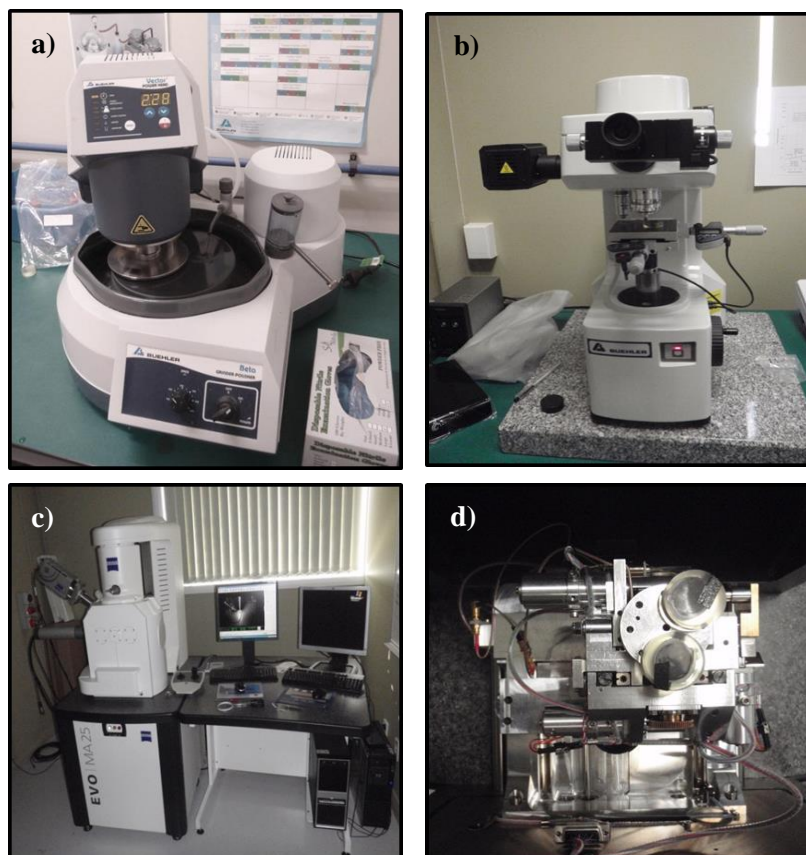


Fig. 5.4. Grinder/polisher used on transverse section of mounted sample (a), microhardness tester (b), scanning electron microscope (c) and mounted sample within the SEM chamber (d).

5.2.4 Statistical analysis

Two way ANOVA and Tukey HSD post-hoc test were used to test differences of the microhardness with two levels (inner and outer) nested with three categorical factors for each estuary surveyed (Statistica version 12, Stastoft Inc).

Trace element concentrations were calculated as mg/kg dry weight excepted for Al, Ca and Fe (%) and Ti (g/kg). The concentrations of trace elements measured in different matrices (surface sediment, tissue, outer and inner shell of cockles) and estuaries were analysed using PERMANOVA based on Euclidean distances (Primer 6, Primer-E ltd). The experimental design considered two factors: matrices (fixed with four levels) and location (fixed with three levels). Principal component analysis (PCA) was used to simplify the data and make it easier to identify the factors that explain most of the variance in the data. Using the mean concentrations of the trace metals and microhardness, the Pearson correlation analysis was used to assess correlation between microhardness and metal concentrations.

5.3 Results

5.3.1 Cockle distribution

The distribution of cockle shells for different size classes revealed large variation among the three different environments (Fig. 5.6). Bureta had the highest total number of cockles found in the 13 cores (412 cockles) representing a density of 2383/m². At this site, the surface of the shell displayed a wide range of colour variation and the amount of dead cockle shell present on the top of the surface sediments was higher than other sites, estimated as ~85% of the surface of the entire cockle bed. Matakana had the second highest total number of cockles in the 13 cores with 371 reported, corresponding to a density of 2146/m². The surface of the cockle shell in this area was mostly “clean” and white, and was associated with less dead cockle shells found on surface sediment (~3% of total area). Rangataua had the lowest total quantity of cockles in the 13 cores with 67

counted, representing a density of 387/m². Cockle shells up to 15 mm were present in greater quantity in Bureta (711/m²) than Rangataua (92/m²) and Matakana (11/m²). However, the abundance of cockles 15-35 mm were greater in both Matakana (2146/m²) than Bureta (1671/m²) and Rangataua (23/m²). Cockles wider than 25 mm and 30 mm were not present in Rangataua and Bureta respectively.

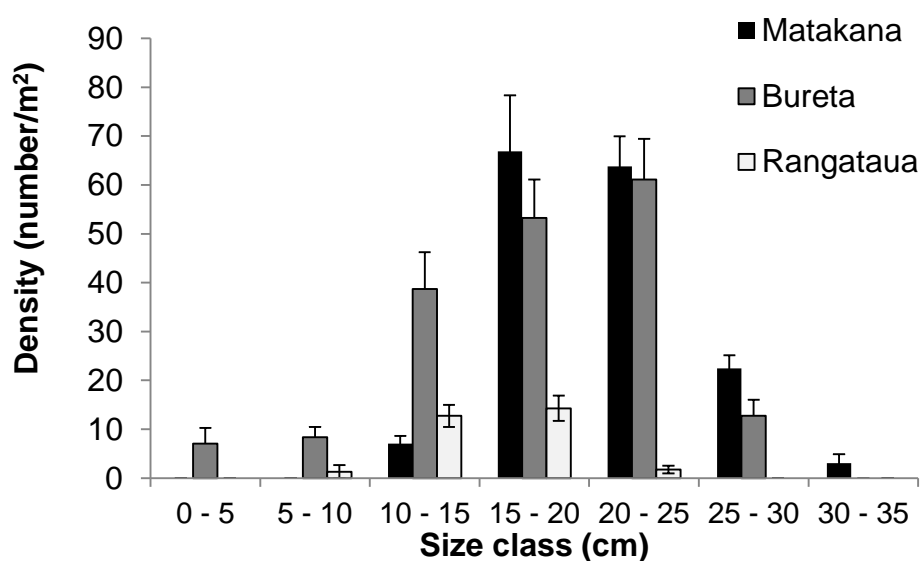


Fig. 5.5. Cockle size class distribution in Matakana, Bureta and Rangataua for 13 core samples.

5.3.2 Microhardness

Microhardness values measured in the inner and outer part of cockle shells displayed variation for different estuaries as well as a function of the part of the shell (Fig. 5.7). There was a significant main effect of location and part of the shells as shown by two-way ANOVA analyses (Table 5.1).

Table 5.1. Two-way ANOVA results for cockle shells microhardness and location

| | SS | DF | MS | F | P |
|--------------------|--------|----|--------|------|-------|
| Estuary | 147527 | 2 | 73763 | 17.2 | 0.001 |
| Shell Part | 206268 | 1 | 206268 | 48.2 | 0.001 |
| Estuary*Shell Part | 7410 | 2 | 3705 | 0.86 | 0.428 |

Microhardness was higher in the inner part of the shell than in the outer part for the three sites. Post hoc comparisons using the Tukey test indicated that the mean score for the outer part of the shell ($M=282.4$, $SD=13.8$) was significantly different than the inner part of the shell ($M=417.8$, $SD=13.8$). Microhardness values were higher in Rangataua, Bureta and Matakana respectively in the inner and outer part of shells. Differences between estuaries in the inner part of the shell were significantly different. Tukey post-hoc test showed significant differences between Matakana and Bureta ($P=0.005$) and between Matakana and Bureta ($P=0.0001$). Tukey post-hoc test showed significant differences between Rangataua and Matakana ($P=0.0001$).

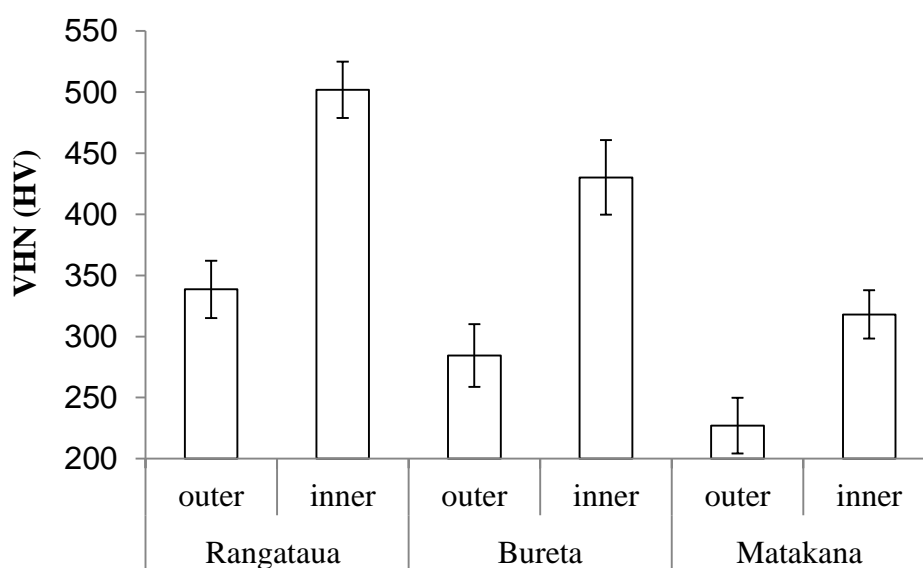


Fig. 5.6. Vickers hardness values (VHN) in the outer and inner part of the cockle shells collected in Rangataua, Waikareao and Matakana. HV as Vickers pyramid number

5.3.3 Elemental analysis

X-ray fluorescence analysis

Trace element concentration measured in surface sediments, the outer and inner part of shells and tissue of cockles were generally higher in Rangataua and Bureta than Matakana (Table 5.2). We found a strong inverse relationship

between the microhardness and the calcium content of the outer shell ($R^2=-0.97$; $P<0.05$). PERMANOVA was applied to the entire set of data and showed significant differences among estuaries ($P<0.001$) and species ($P<0.001$). In surface sediment, most trace elements were accumulated in Rangataua, Bureta and Matakana in order of magnitude. Ti, V, Fe, Cu, Zn and W concentrations were higher in Rangataua; Cr, As, Rb, Ba and Pb in Bureta, and Al, Ni, and Br were higher in Matakana. Ca content in surface sediment was higher in Bureta (2.4%) than Rangataua (2.2%) and Matakana (1.6%). Most of the variation in surface sediment was not significant due to a low number of replicates.

The elemental composition of shells displayed differences between the inner and outer part of the shell. Al, Ti, V, Cr, Fe, Cu, Zn, As, Br and Rb concentrations were higher in the outer part of the shells than in the inner part regardless the location. Ba concentration was higher in the outer part of the shell only in Rangataua. Ca, Ni, Cd, W, Hg and Pb concentrations were higher in the inner part of the shell for the three estuaries as well as Ba for Bureta and Matakana. These results were statistically significant for Al, Ca, Ti, V, Fe, Zn, Br, and Rb (ANOVA; $P<0.05$) in Rangataua, for Al, Ca, Ti, V, Cr, Fe, Rb, Ba, W, Hg and Pb (ANOVA; $P<0.05$) in Bureta and for Al, Ca, Ti, V, Cr and Fe (ANOVA, $P<0.05$) in Matakana. Trace element concentrations in the outer part of shells showed significant differences among the three estuaries (PERMANOVA; $P<0.005$). Al, Ti, Fe, Cu, Zn, As, Br, Rb, and Ba concentrations in the outer part of shells were higher in Rangataua than in Bureta and Matakana. Analysis of variance showed significant differences for Ti, Fe, Cu, Zn, Rb and Ba ($P<0.05$). Trace element concentrations in the outer shell were higher in Bureta than Matakana except for Ca, Ni, Zn and Hg. Trace element concentrations in the inner part of the cockle shell displayed significant differences between estuaries

(PERMANOVA; $P < 0.01$) except between Matakana and Rangataua. Generally, trace metals were accumulated in greater concentration in Bureta which was only significant for Ti (ANOVA; $P < 0.05$). Al Cr and Zn concentrations in the inner part were higher at Rangataua while Ca and Ni concentrations were higher in Matakana.

Trace elements in tissues were higher in Bureta than in Rangataua and Matakana. Ti, V, Cr, Ni, Zn, As, Br, Rb, Ba, W and Pb were higher in Bureta, statistically significant for Ti, V, Br, Ba and W (ANOVA, $P < 0.05$). Ca and Cu were significantly higher in Rangataua (ANOVA, $P < 0.05$). Finally, Cd and Hg were higher in Matakana.

Table 5.2. Trace element concentration (mg/kg) in sediment, outer and inner part of the shell and tissue of cockles collected in Rangataua, Bureta and Matakana estuaries; ND: no detectable.

| Matrice | Location | Al (%) | Ca (%) | Ti (g/kg) | V | Cr | Fe (%) | Ni | Cu | Zn | As | Br | Rb | Cd | Ba | W | Hg | Pb |
|------------------|-----------|-----------|-----------|--------------|-------|------|-----------|------|------|-------|------|-------|------|-----|-------|-----|-----|------|
| surface sediment | Rangataua | 7.4 | 2.2 | 11.0 | 170.0 | 19.3 | 3.6 | 11.4 | 14.0 | 80.8 | 4.1 | 48.4 | 48.8 | ND | 122.4 | 5.5 | ND | 14.0 |
| | se | 0.4 | 0.5 | 4.2 | 63.3 | 1.6 | 0.4 | 2.1 | 2.0 | 7.3 | 1.4 | 12.4 | 8.6 | ND | 34.5 | 0.8 | ND | 2.7 |
| | Bureta | 6.9 | 2.4 | 3.7 | 67.1 | 20.1 | 2.6 | 7.2 | 6.4 | 69.7 | 5.9 | 101.9 | 66.6 | ND | 138.3 | 5.3 | ND | 15.2 |
| | se | 0.4 | 0.1 | 0.3 | 16.0 | 0.8 | 0.1 | 0.5 | 0.9 | 1.5 | 0.1 | 4.9 | 2.4 | ND | 16.3 | 0.3 | ND | 0.5 |
| | Matakana | 7.5 | 1.6 | 4.9 | 124.0 | 18.7 | 3.0 | 9.2 | 10.4 | 78.2 | 5.6 | 127.6 | 48.0 | ND | 80.9 | 5.4 | ND | 14.5 |
| | se | 0.3 | 0.1 | 0.6 | 36.7 | 0.9 | 0.1 | 0.6 | 0.2 | 2.8 | 0.1 | 4.4 | 6.1 | ND | 19.0 | 0.2 | ND | 1.1 |
| shell (outer) | Rangataua | 2.4 | 27.4 | 0.7 | 31.3 | 7.4 | 0.8 | 5.6 | 3.7 | 26.2 | 1.6 | 46.4 | 4.1 | ND | 12.6 | 0.0 | 0.4 | 3.3 |
| | se | 0.5 | 0.6 | 0.2 | 12.4 | 3.0 | 0.4 | 2.1 | 2.1 | 3.9 | 1.6 | 6.5 | 1.3 | ND | 1.2 | 0.0 | 0.4 | 0.7 |
| | Bureta | 1.6 | 28.7 | 0.4 | 28.3 | 7.3 | 0.2 | 4.2 | 0.0 | 14.1 | 0.2 | 53.5 | 2.0 | ND | 6.1 | 2.1 | 0.5 | 3.3 |
| | se | 0.2 | 2.7 | 0.1 | 3.6 | 1.6 | 0.0 | 0.8 | 0.0 | 4.6 | 0.2 | 24.9 | 0.5 | ND | 2.0 | 0.5 | 0.2 | 0.3 |
| | Matakana | 1.5 | 30.4 | 0.1 | 9.0 | 6.3 | 0.1 | 7.7 | 0.0 | 20.2 | 0.0 | 34.1 | 1.5 | ND | 5.9 | 2.6 | 1.0 | 3.1 |
| | se | 0.1 | 1.6 | 0.0 | 1.2 | 1.2 | 0.0 | 1.0 | 0.0 | 4.9 | 0.0 | 6.7 | 0.2 | ND | 0.6 | 1.2 | 0.4 | 0.5 |
| shell (inner) | Rangataua | 0.9 | 41.5 | 0.2 | 0.6 | 3.5 | 0.0 | 9.9 | 0.0 | 12.7 | 0.0 | 10.5 | 1.0 | 0.3 | 8.9 | 5.8 | 1.0 | 3.4 |
| | se | 0.2 | 1.3 | 0.1 | 0.6 | 2.2 | 0.0 | 3.2 | 0.0 | 2.4 | 0.0 | 3.5 | 0.2 | 0.1 | 1.6 | 1.2 | 0.5 | 0.7 |
| | Bureta | 0.6 | 38.5 | 0.2 | 3.4 | 3.3 | 0.0 | 5.9 | 5.7 | 8.4 | 0.0 | 17.2 | 1.2 | 1.4 | 12.7 | 6.4 | 2.1 | 4.6 |
| | se | 0.2 | 3.2 | 0.0 | 0.8 | 1.9 | 0.0 | 1.3 | 0.0 | 1.7 | 0.0 | 8.1 | 0.1 | 0.4 | 1.7 | 0.5 | 0.3 | 0.3 |
| | Matakana | 0.8 | 44.8 | 0.1 | 0.0 | 0.0 | 0.0 | 8.9 | 0.0 | 9.8 | 0.0 | 24.5 | 1.0 | 0.5 | 8.3 | 5.5 | 1.7 | 4.2 |
| | se | 0.1 | 4.5 | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 | 0.0 | 1.7 | 0.0 | 16.5 | 0.2 | 0.1 | 2.3 | 0.9 | 0.2 | 0.2 |
| tissue | Rangataua | 0.5 | 4.6 | 0.1 | 2.0 | 1.9 | 0.1 | 24.4 | 27.0 | 100.4 | 17.6 | 245.5 | 6.6 | 0.1 | 4.6 | 2.9 | 0.4 | 2.4 |
| | se | 0.1 | 1.3 | 0.0 | 0.8 | 0.5 | 0.0 | 3.5 | 6.6 | 9.9 | 3.6 | 66.9 | 0.3 | 0.1 | 1.8 | 0.9 | 0.3 | 0.5 |
| | Bureta | 0.5 | 1.5 | 0.2 | 3.6 | 2.6 | 0.1 | 30.2 | 7.1 | 98.8 | 32.2 | 545.9 | 7.4 | 0.1 | 8.9 | 5.3 | 0.7 | 3.3 |
| | se | 0.1 | 0.1 | 0.0 | 1.8 | 2.1 | 0.0 | 2.6 | 1.2 | 4.4 | 2.6 | 15.3 | 0.3 | 0.1 | 1.0 | 0.3 | 0.2 | 0.3 |
| | Matakana | 0.5 | 2.3 | 0.1 | 1.3 | 0.9 | 0.1 | 23.8 | 7.3 | 86.4 | 16.8 | 332.5 | 5.7 | 0.2 | 7.2 | 4.5 | 0.8 | 3.3 |
| | se | 0.0 | 0.6 | 0.0 | 0.6 | 0.5 | 0.0 | 7.9 | 1.9 | 5.7 | 5.0 | 35.4 | 0.4 | 0.1 | 1.1 | 0.8 | 0.3 | 0.4 |

A PCA combining the results from sediment, the outer and inner part of the shell and tissues of cockle grouped the trace metals content into four distinct groups, with the first two PCA components explaining 70% of total variation (Fig. 5.8). The first group (Ba, Fe, Pb, Ti, V, Al and Cr) was mostly associated with surface sediment samples and the outer and inner part of the shell in decreasing order of concentration. The second group (Hg and Cd) was mainly associated with the inner part of the shell. The last group (Ni, Br, As, Cu, Zn and W) was associated with bivalve tissues composition.

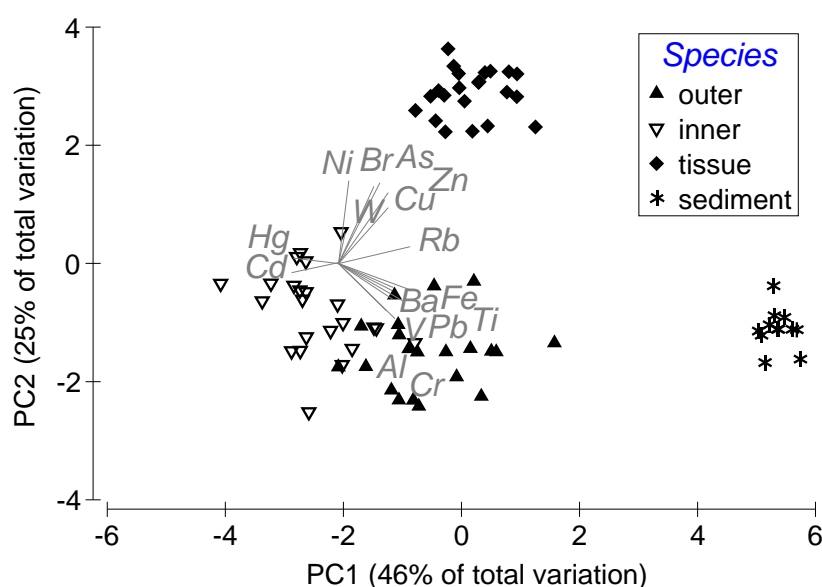


Fig. 5.7. Ordination plots of the primary component analysis of metal concentrations in the tissues, outer/ inner part of shells of cockle and surface sediment in Rangataua, Bureta and Matakana combined.

Scanning electron microscopy with x-ray microanalysis (SEM/EDS)

Trace element concentrations measured by SEM/EDS were very variable for shells collected within the same estuary as well as different positions within the same shell. Statistical significance has been tested with PERMANOVA examining the variation of trace elements within the cockle shell (inner, middle and outer) for each estuary. Variation of trace elements between Matakana and Bureta were not significant (PERMANOVA; $P > 0.05$). However, trace elements

measured in cockle shell collected in Rangataua were significant (PERMANOVA; $P < 0.05$). A PCA analysis plot was used to visualise the elemental composition of the inner, middle and outer part of shells for each estuary, demonstrating the high degree of variability (Fig. 5.9) and the resultant low level of confidence in the explanation of such variabilities.

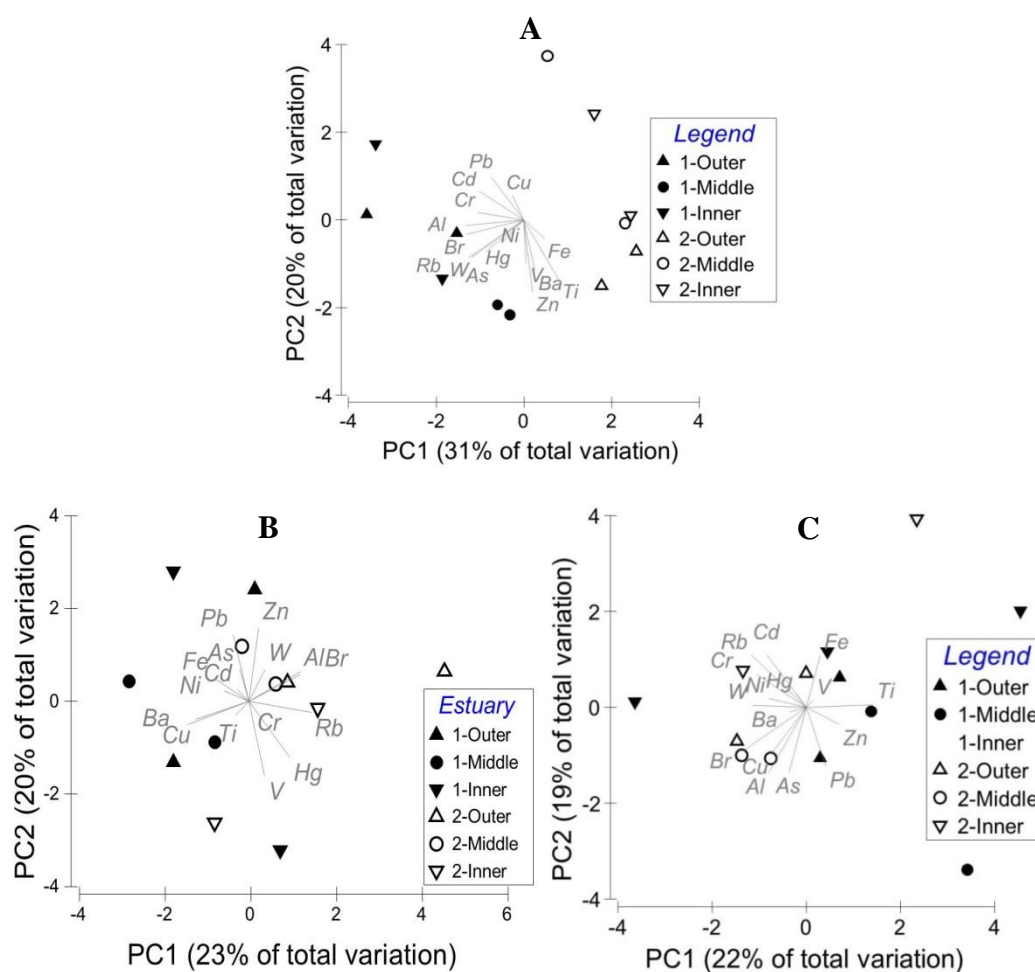


Fig. 5.8. Ordination plots of the primary component analysis of metal concentrations in the outer, middle and inner part of cockle shells collected in Rangataua (A), Bureta (B) and Matakana (C).

X-ray elemental mapping

X-ray elemental mapping was carried out to examine trace metal distribution within the cockle shell at different magnitudes. Fig 5.10 (a, c) shows SEM micrographs at a magnitude of 60 μm and 4 μm respectively. At a magnitude of 60 μm (Fig 5.10 b), Pb concentrations were heterogeneous. At higher magnification, the ultrafine embedded particles confirmed a homogenous distribution of Pb throughout the shell, as shown in Fig 5.10 (d). Distribution of

As was also examined at different locations within the same shell for a same magnitude (Figs. 5.11a-h). The distribution of As molecules were heterogeneous on the surface of cockle shell. As concentrations observed were higher in Fig 5.11 (e, h) than Fig 5.11 (f, g).

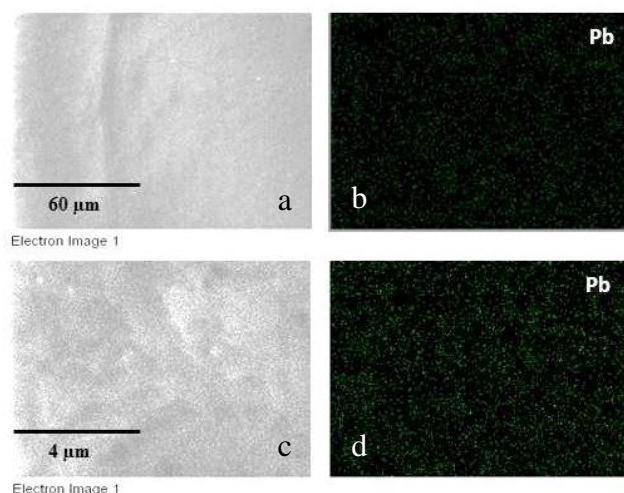


Fig. 5.9. SEM micrographs (a-c) and corresponding x-ray elemental mapping (b-d) of Pb content at 60 µm and 4 µm in the cross section of *Austrovenus stutchburyi* ventral margin.

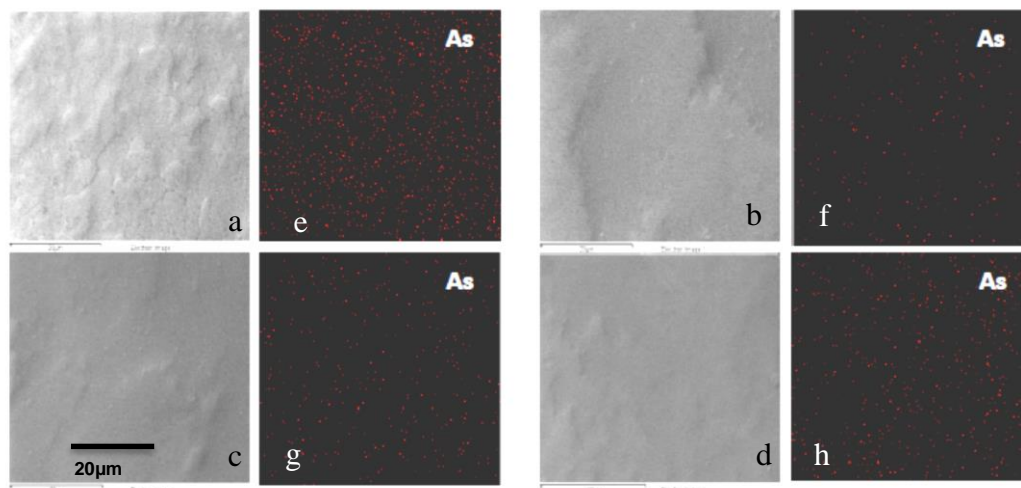


Fig. 5.10. SEM micrographs (a-d) and corresponding x-ray elemental mapping (e-h) of As content at 20 µm in the cross section of *Austrovenus stutchburyi* ventral margin

5.4 Discussion

5.4.1 Microhardness: a “novel” environmental parameter

In the past few decades, the increase of pollutants from the production, disposal and release of waste into the natural world has been of growing concern (Simon, 1971; Cifric, 1988; Clements, 2012). The general health of estuaries in

particular is of fundamental importance, as it will determine the future well-being of species inhabiting the ecosystem including humans. Furthermore, estuaries can be considered as providing a snapshot of historical pollution events that *en masse* could affect the present biological, physical and chemical state. Environmental indicators such as water clarity, temperature and pH have been developed to understand the state of such environments. The elaboration of environmental parameters has been constantly developed and tested to permit a more accurate and relevant understanding of the fate and effects of pollutants such as, for example, the elaboration of safety guidelines for metals and other contaminants (Long, *et al.*, 1995; ANZECC, 2000). The recent use of nitrogen and carbon isotopic ratios permitted tracking of the potential sources of nitrogen loading responsible for eutrophication (Rogers, 1999; Gammons, *et al.*, 2011; Hartland, *et al.*, 2011; Rogers, *et al.*, 2012), see also Chapter 4. More recently, the isotopic ratio of trace metals has been developed to find sources and sinks of mining pollution and other metallic related land use activities (Allan *et al.*, 2015; Félix *et al.*, 2015; Resongles *et al.*, 2015). However, there are constraints for these methods as for example the number of contaminants considered in ecotoxicology studies are limited (ANZECC, 2000)(Chapter 2). The estimation of bulk concentration of metals in sediment and the water column is of little use in ecotoxicology studies unless supported with temporal information, and hence the development of new approaches such as the use of passive sampler (e.g. diffuse gradient in thin film) has advanced understanding in metal speciation and associated bioaccumulation. The relevance of laboratory studies to *in situ* exposure to contaminants remains uncertain due to the high level of complexity found in natural environment however necessary such tests are in the development

of safety guidelines for environmental protection (Liber *et al.*, 2007; Belzunce-Segarra *et al.*, 2015).

With these monitoring options and limitations in mind, we developed a novel metric to give a more accurate understanding of anthropocentric impact. This study aimed to test if bivalve shell microhardness could be used as an indicator to describe the level of chemical contamination between urban and semi-oceanic environments. Rangataua and Bureta estuaries have been assessed as some of the most degraded areas in the Tauranga Harbour in terms of metal pollution, with Rangataua showing higher accumulation of toxic elements (Chapters 2 and 4). Sedimentary analysis of trace elements confirmed a higher accumulation of “toxic” trace elements in Rangataua, Bureta and Matakana in order of decreasing magnitude.

Microhardness measurements have been made in the inner and outer zones of the shell collected from bivalves from three sites across a pollution gradient. Microhardness was higher in the inner part than the outer part of the shells for all sites, confirming past research examining variation of microhardness between the inner and outer shell zones (Zhu, *et al.*, 2006; Yang, *et al.*, 2011). These differences were well known and have been attributed to a change in the calcium microstructure of the shell, as indicated in Fig 5.1 (Pérez-Huerta, *et al.*, 2007). Additionally, differences in the elemental composition of cockle shell were investigated to assess any potential effects on microhardness. We found that most metals were accumulated in the outer shell (Al, Ti, V, Cr, Fe, Cu, Zn, As, Br and Rb) therefore differences of microhardness between inner and outer regions appears due to differences in microstructure rather than other parameters such as elemental composition.

The novel element of this present study was to investigate if microhardness varied in locations with differing environmental conditions. We found an increase of microhardness linked with environmental degradation in both, the inner and outer part of the shell. The microhardness in the inner and outer shells was significantly higher in Rangataua and Bureta than Matakana with the highest values recorded in Rangataua, thus suggesting an increase in microhardness with increasing pollution index. Elemental composition of the shell needed to be assessed to understand if the variations of microhardness were due to changes in the elemental composition of cockle shells. The elemental composition of the shell was found to vary with locations, in accordance with previous studies which examined the chemical composition of shell as an environmental fingerprint (Purchase & Fergusson, 1986; Ahmad, *et al.*, 2013; Pourang *et al.*, 2014). Ca concentrations in both outer and inner parts of cockle shells were higher in Matakana than Bureta and Rangataua. In parallel, the decrease of Ca content (soft matrix) was correlated with an increase of “harder” oxide elements or impurities in Bureta and Rangataua. In the outer part of the shell, Al, Ti, V, Cr, Fe As, Br, Rb, Ba and Pb concentrations were lower in Matakana than Bureta and Rangataua. In the inner shell, Ti, V, Cr and W concentrations were lower in Matakana than Bureta and Rangataua. This demonstrated that the outer shell was a lot more sensitive to its local chemical environment probably due to direct adsorption from the water column influenced by the sedimentary composition (Zuykov *et al.*, 2012).

In laboratory experiments, Almeida *et al.* (1998) demonstrated that increased exposure of *Crassostrea gigas* to Pb was linked with higher accumulation of Pb within the shell and a decrease of Ca concentrations. Nuñez, *et al.* (2012) measured elemental composition in limpet shells collected from

heavily polluted, lightly polluted and unpolluted estuaries. They found similar results to those presented here with higher Ca concentration in shell in less polluted estuaries and lower Ca concentration for the most contaminated estuaries. Lower microhardness in Matakana could be attributed to higher Ca content and a lower concentration of other trace elements. Inversely, for Bureta and Rangataua Ca content was lower due to an increase of other trace elements as mentioned previously. Ca is ranked as a soft material with a microhardness value lower than most of the other elements found in the periodic table (Pearson, 1990, 1992; Kaya & Kaya, 2015). In this section, we confirmed past research on microhardness in bivalve shells which demonstrated that microhardness was higher within the inner area of the shell due to complex cross lamellar microstructure. The novelty of this research included the variation of microhardness values (in both inner and outer shells) with different environments characterised by different pollutions stress and Ca content within the shells.

5.4.2 Origins and fates of trace elements in cockle shells

The comparison of trace elements between the ambient environment (sediment) and the different anatomy of the cockle (tissue versus shell) improved our understanding of the biological response of this animal toward chemical stress. Three different groups of metals could be distinguished when concentrations in sediment, cockle tissue and shell are compared to indicate trends in retention. Ba, Fe, Pb, Ti, V, Al and Cr were mainly associated with surface sediment; Ni, Br, As, Zn, Cu, Rb and W with tissues; and Hg and Cd associated with the inner part of the shell. Higher concentration of trace elements in tissues of cockle collected at Bureta could be explained by higher fluctuations in the water column as tissues are more sensitive to short term variation (Lares *et al.*, 2005). We found that the elemental composition of the outer part of the shell was more closely related to

sediment than the inner part. The outer part of the shell (lacking metabolic activity) is in direct contact with the sediment which could explain their similarities due to direct adsorption from the external medium. However, the elemental content in the inner part of the shell was slightly more related to tissues than the outer part and showed stronger affinities with Hg and Cd. The inner part of the shell is in close contact with the tissue, and hence can integrate trace elements only from the extrapallial fluid (organism metabolism) (Bourgoin & Risk, 1987; Lares, *et al.*, 2005). This means that all the components incorporated in the internal shell layer are a result of the animal's metabolic activity. It is feasible that cockles depurate Cd and Hg from their tissues by the displacement of these elements into the inner part of the shell. The analysis of Hg and Cd content in the inner shell appeared to be a good indicator when assessing Hg and Cd toxicity in ecological studies. Previous studies showed that clams (*Ruditapes philippinarum*) were able to decrease elemental concentration in their tissues during depuration of Fe, Pb, Cu, Rb, Br, Hg, Cd and As while depuration pathways were not specified (Anacleto *et al.*, 2015). In the present study, higher accumulation of Pb was found in the inner part of the shell than outer part. Similar studies of Pb deposition have indicated the majority of accumulated Pb is bounded into the inner part of the shell (nacreous matrix) via a direct isomorphic substitution in CaCO_3 in its transfer across the mantle via ion flux (MacFarlane *et al.*, 2006).

The observation at the microscopic scale by SEM/EDS analysis showed that elemental variation in the cockle shell was very complex. This was demonstrated by the presence of ultrafine particles of Pb at 4 μm as well as a heterogeneous accumulation of As in different parts of the shell.

5.5 Conclusion

This study examined the biological, chemical and mechanical properties of cockles found in Tauranga Harbour. Microhardness has been successfully used to fingerprint the chemical budget of estuaries. Microhardness was higher in Rangataua than Bureta and Matakana, with lowest values found in Matakana. We demonstrated that microhardness increased with a decrease of Ca content in the shell due to an increase of oxide impurities. The less polluted site had higher Ca content in the shell corresponding to lower values of microhardness.

The second aim was directed at developing our understanding on the biological response of cockles to chemical stress. We found that some trace elements were prone to higher bio-accumulation explained by elemental speciation (Chapter 3). Some other elements were more concentrated in surface sediment rather than in tissues due to the efficiency of depuration or a low affinity to be absorbed by tissues. The outer part of the shell were more associated with the sediment than the inner part due to direct adsorption. Finally, Cd, Pb and Hg showed a different pattern with higher accumulation in the inner part of the shell than in the outer, probably due to the toxic nature of these elements (depuration).

This research has highlighted the use of microhardness as a new environmental indicator. We demonstrated that microhardness values were not only associated with changes in microstructure, but fluctuations of Ca and trace elements in estuarine systems, affected by different anthropocentric inputs. In the future, this new indicator could be a robust parameter for the detection of environmental changes in response to pollution and climate change events. It could be of use by the aquaculture industry as a robust index when small variations in chemical and morphological proprieties of shells can have important

impact on species growth and production. A graphical abstract is provided in Fig 5.12.

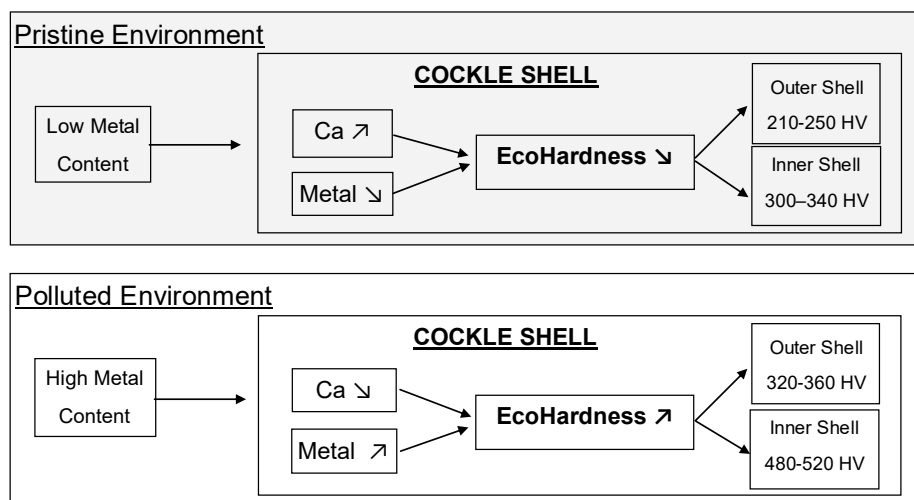


Fig. 5.11. Graphical abstract of the relationship between elemental content and microhardness.

6 Chapter Six

Conclusion

6.1 General Discussion and Recommendations

In the last few decades, nutrients (e.g. nitrogen and phosphorus) and toxic trace elements (e.g. lead and cadmium), have been deposited in high concentrations in estuaries worldwide (Bryan & Gibbs, 1983; Han, *et al.*, 2013; Morelli & Gasparon, 2014; Diop *et al.*, 2015; Yang, *et al.*, 2015b) including New Zealand estuaries (Burggraaf, *et al.*, 1997; Stewart *et al.*, 2014). Sources of contamination have been linked with natural background geology in some instances, but are often reflecting human activities from mining, oil exploitation, uncontrolled use of chemical fertilisers and urban development linked particularly with sewage discharge (Duan, *et al.*, 2015; Othmani, *et al.*, 2015; Yang, *et al.*, 2015b). The occurrence of trace metal contaminants in food (e.g. Cd, As and Pb) can affect food safety when consumed and can lead to significant deleterious health effects (Clarkson, 1993; Järup & Åkesson, 2009; Thomas, *et al.*, 2009).

Environmental managers control activities occurring on the land to promote a sustainable environment and have responsibilities to develop, implement and monitor environmental strategies, policies and programmes. These are commonly based on economic, social and scientific evidence. New Zealand ecosystems will only be adequately protected if researchers are designing monitoring plans, to integrate a wide range of pollutants in sediment, water and biological species at different locations. Many contaminants are not under regulatory action, and most of them are not screened in quality assessment

including emerging contaminants such as new pharmaceutical compounds that are being released from sewage systems (Stewart, *et al.*, 2014).

Tangata Whenua defined as environmental guardians, are actively interested in the state of the local environment due to their strong cultural heritage. Consultation with Tangata Whenua under the Resource Management Act 1991 (RMA) is a legal requirement in some circumstances. They believe that their wellbeing and life force are directly associated with the state of the environment as an interdependent relationship exists between humans and the natural world. Additionally, Tangata Whenua are the largest consumer of seafood especially for species collected in estuaries; however the biota has not been comprehensively screened for pollutants in the Bay of Plenty (and indeed other regions), and information on the health of seafood are not systematically integrated into management plans (Personal communication; Bay of Plenty, Greater Wellington and Canterbury Regional Councils, Auckland City Council).

The objective of this thesis is to understand the sources, fates and effects of trace metals and nutrients (nitrogen and phosphorus) in complex harbour systems utilising Bay of Plenty estuaries as representative case studies. A different approach compared to past studies has been achieved to support new interpretation and provide new insight for environmental management. Sedimentary and biota samples were collected from five estuaries to examine fluctuations in elemental content between and within estuaries. This study was the first attempt to investigate the coupling of sedimentary and water processes in Rangataua, Waimapu, Waikareao, and Tuapiro sub-estuaries in Tauranga Harbour as well as in Ohiwa Harbour (a relatively uncontaminated ‘control’ estuary), together with resultant interactions with benthic species. This research supported

by numerous analytical technologies reported for the first time $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ values and labile chemical (diffuse gradient ‘in thin film’ units) in water with sedimentary and biota analyses. Furthermore, a new environmental parameter reflecting levels of pollution by using cockle shell hardness was developed as a repeatable environmental monitoring technology, that is argued to provide a valuable new tool for future use by environmental agencies and aquaculture businesses. In this research, we worked in collaboration with a local iwi, Nga Potiki, which was interested in understanding the impacts of treated wastewater seepage on mud snails in Rangataua estuary.

In Tauranga Harbour, research on estuarine contamination from both urban and rural sources has been scarce and mainly limited to surface sediment studies reporting general trends for the whole estuary without information on sources and providing limited knowledge on effects on biota (Park, 2003; Ellis, *et al.*, 2013; Park, 2014). The Bay of Plenty Regional Council carries out contaminant surveys for the Tauranga and Ohiwa Harbour to monitor concentrations of 8 trace metals (As, Cd, Cr, Cu, Pb, Hg, Ni and Zn) and other organic compounds (e.g. PAH) in surface sediment. They found no increase in trace elements with time except for the Waimapu Estuary; however, the first report on contaminants was made 16 years ago and repeated every 6 years; therefore historical data collected in these surveys was too small to permit any statistical comparison. They found that lead and zinc were higher in areas with the highest level of urban and commercial development (Park, 2014). In future research, historical data could be collected using sedimentary cores and a range of techniques such as stable isotope dating and trace metals analysis (Dickinson, *et al.*, 1996; Hosono, *et al.*, 2010; Wang, *et al.*, 2015a). Measuring environmental

concentrations of these contaminants in surface sediment is only the first step in understanding the potential effects that pollutants may have on the environment.

In the present study, surface sediment was a useful indicator to identify trace metals enrichment between estuaries. The advantage to sample sediment is its availability to be collected while most biological species are influenced by ecological zonation therefore not always present at all locations. Ohiwa Harbour (the control site) representative of a rural environment with moderate land activities showed lower concentrations of metals. Trace elements in surface sediment showed little variation between urban estuaries most likely due to direct interconnection supported by their proximity with each other and associated water exchange. However, major differences between sites within the same estuary were found for all trace metals and nutrients with higher concentration near freshwater input. For example, the Waimapu River contributed to the discharge of Al, Pb and U as well as nitrogen and phosphorus while the Waitao River contributed to higher discharge of Al, Ni and TN. Stormwater inputs contributed significantly in the loading of Al, Cu, Zn, Cd, Pb and U (Chapter 2) as well as nitrogen and phosphorus (Chapter 3). Zn, Pb and Cu content near the stormwater outlet had similar values recorded than the most degraded sites in the Auckland region where sedimentary quality guidelines were exceeded (Stewart et al., 2014). DGT was not used in stormwater discharge areas to assess the concentration of elements which are bio-available, as water was only present during high tide. However, a future study displaying DGTs within stormwater drains for a short period (e.g. 3 hours) could record the contribution of the drain systems towards the general bioavailable trace elements budget in estuaries. Research undertaken in the Hatea River catchment and estuary showed similar results with higher concentration of Fe, Mn, Cu, Pb, Zn and As in stormwater compared to stream

tributaries (Webster *et al.*, 2000). From this research, we found that combining different indicators for the measurement of ambient concentration (surface sediment, water and DGTs) provided complementary results and permitted improvement of our interpretation of the effects of contaminants in estuarine systems.

Sea lettuce has been previously used as a bio-indicator to assess Zn and Cu contamination in the Otago Harbour (Brown, *et al.*, 1999). The authors found that the Otago Harbour was not contaminated and representative of a pristine environment. In the present study, Cu level in sea lettuce tissue was in the range of 5-20 ppm representative of moderate contamination (Cu contaminated environment ranged between 14-130 ppm); however, the concentration in Tauranga Harbour was 7 times higher than the Otago Harbour. Higher concentration of Cu in sea lettuce tissues was found near the wastewater treatment plant and the outlet of the Waimapu River. The lowest concentration of Cu in sea lettuce tissue was found in Tuapiri. Zinc concentration (40-907 ppm) in sea lettuce tissue showed important differences with location. These results were 24 to 34 folds higher than the Otago Harbour and therefore representative of very contaminated sites with higher concentration near the Waitao River outlet (Rangataua), the Waikareao stormwater drain and sulphur point. Elevated zinc concentration in sulphur point was recorded in cockle tissue with value similar to those found in oyster tissue (Luoma, *et al.*, 2008). Elemental enrichment near the stormwater outlet was detected in seagrass blades with higher concentration of As, Zn, Cu, Cr and Al (chapter 2) as well as nitrogen and phosphorus (chapter 4). Sedimentary and seagrass isotopic values displayed distinct signatures near the stormwater effluents which could be used in future impacts studies. This finding will be a useful tool for environmental managers in the future detections of

stormwater impacts. In this research, we showed that the use of a range of biomonitors, especially primary producers, allowed recognition of trace metals and nutrients enrichment in estuaries as well as the importance of freshwater inputs. The assessment of contaminants by collecting and analysing surface sediment can be reinforced by the analysis of primary producers which have more direct, simple, and predictive accumulation pathways than consumers. However, we did not find any clear correlation between ambient levels of trace metals and biota within estuaries, which showed the difficulties in the prediction of metal accumulation in species based on surface sediment analysis only. While trace metals have been routinely measured in surface sediment, biological species that are consumed by the public should be integrated in future monitoring management plan.

In this research, we were able to show that coastal water was different to estuarine water with elemental composition in offshore water lower than results measured in estuaries. Most importantly, however, measurements of total and dissolved metal concentration provided an assessment of metal present, not of that portion which is bioavailable. Time integrated measure of metal supply over weeks have been recorded with DGT monitors to measure the concentration of metals which are bio-available, as opposed to total metal present. In this study, safe consumption guidelines were exceeded for Pb and Cd in estuarine species, but comparisons with other studies for the same location were not feasible as the Bay of Plenty Regional Council focussed on the assessment of non estuarine species. In general, metal accumulation in estuarine species was strongly influenced by the feeding modes and habitat usage of species, with bulk sediment composition being of secondary importance. Trace element enrichment in biota followed consistent hierarchies within individual species that were indicating a

high degree of uniformity in accumulation mechanism, despite the high spatial variability in the sediments.

However, the diet of mud snails was associated with sewage input as tracked by stable isotopes analysis. The detection of treated wastewater characterised by higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in sediment and mud snails permitted monitoring of the impacts of wastewater discharges as far as 1 km away from the ponds, and was associated with the input of phosphorus and cadmium rather than nitrogen. The comparison of different analytical techniques (e.g. trace elements and stable isotopes) improved our interpretation in the effects of pollution sources in estuarine environment. This finding was valuable for both the City Council concerned and Nga Potiki which are now working in collaboration in the development of remediation strategies. While $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses supported fundamental understanding of the localisation of pollution sources (e.g. wastewater and fertilisers), it appeared that further research measuring the isotopic ratio of metals needs to be considered in the future. For example, Pb isotope ratios have been used as an indicator of pollution sources such as coal, vehicle emissions and Pb ores (Sun *et al.*, 2011; Ningjing *et al.*). Environmental managers are now supported with a relevant baseline for estuaries which will permit direct comparison with future studies. Due to the heterogeneous distribution of trace elements in the biota, it is advised to collect seafood further away from any storm water or freshwater outlets.

This research reports the development of a new environmental parameter “EcoHardness” by the measurement of microhardness in cockle shells. Microhardness has been previously measured in bivalve shell (inner and outer) and was associated with a change in the shell microstructure (Yang, *et al.*, 2011).

In this research, microhardness was higher in the inner part of the shell than the outer shell, confirming past studies. However, the measurement of microhardness in different estuaries with specific pollution stress had never been made before. Cockles were sampled in Matakana, Bureta and Rangataua in increasing order of pollution, as demonstrated in previous chapters. Degradation of the environment was reflected in cockle distribution with larger cockles found in the most pristine environment (Matakana). Microhardness increased in urban estuaries due to a decrease in Ca concentration associated with an increase of trace metals. This new indicator reflected the morphological characteristics of cockle shells as a response to changes in their chemical composition as well as providing an indication of chemical fluctuation in estuaries. In the future, we would recommend the analysis of microhardness as an indicator of historical contamination by continuous grinding and analysis of the shell, as well as the development of a microhardness baseline in different species focusing on commonly harvested species in aquaculture such as mussels and oysters.

Overall, this study provided new baselines on trace element concentrations in a species assemblage (trophic cascade) not previously screened permitting direct comparison with new data collected in the future. For example, mud snails, seagrass and sea lettuce have not been analysed in the past by the Bay of Plenty, Wellington and Canterbury Regional Councils as well as the Auckland Council, and this set of data will be beneficial to understand various pathways of contaminant uptake. Therefore, the use of a suite of bioindicators will integrate a relevant range of contaminant effects across representative estuarine species, and should allow the identification of specific contaminant sources. For example, sea lettuce samples will reflect metal only available in suspended particles, as opposed to mud snails and seagrass tissues reflecting the elemental concentration

in sediment. In this research, we provided new information to support environmental managers and provided a wide range of guideline and feedback to improve current practices. We found that various methods have been used in the preparation, analysis and reporting of data causing different interpretation on chemical abundance and not always permitting comparison between different studies. For example, the Auckland Council has been using two different methods in the acidification process for metal extraction in the same report. Fine sediment fraction (<63 μm) extraction was via cold 2 M HCl and developed by NIWA while the medium sediment fraction (<500 μm) was acidified using a stronger digestion of HNO_3/HCl (USEPA Method 200.2). In this research, we chose to use the same methodology in the recovery of trace metals by acid extraction to limit variability not due to environmental differences. Additionally, we showed that metal accumulation was different between small and large mud snails and similar results have been found previously in New Zealand cockle (Marsden, *et al.*, 2014). When comparing biological species in environmental research, the size or age of organisms has to be carefully considered depending on the research question. For example, comparing mud snails of same size will permit examination of location effects while comparing mud snails of different sizes will allow the detection of the fraction of metal recently deposited. Some research analysed whole organisms prior metal analysis while other studies elected to depurate animals. Marsden *et al* (2014) demonstrated that concentration of Fe, Mn, Ti and V in cockle tissues decreased significantly after depuration indicating a high presence of these elements in the gut. In this research, we choose to not depurate mud snails and cockles prior analysis to replicate the method that seafood gatherers would prepare the seafood before to be consumed.

This study has demonstrated the benefits of examining a relevant range of estuarine organisms that co-occur yet have different ecological functionalities. It has demonstrated the benefits of combining a range of new technologies to understand effects of complex contaminant mixtures sourced from both urban and rural catchments. Finally, this research has identified a novel mechanism based on the microhardness of bivalve shells that integrates across a range of effects brought about by dynamic pollution incursions. The overall outcome of this research is to improve estuarine ecosystem health through the provision of improved understanding, knowledge, analysis and enhanced indicators for estuarine ecosystems.

6.2 A Summary of Scientific contributions

- Characterisation of the ecological state of Bay of Plenty estuaries by the analysis of toxic and essential trace elements in sediment, water and biota
- Identification of the main sources of contaminants such as rivers and stormwater drains
- First baseline of elemental content and stable isotope ratios in water, seagrass, mud snails and sea lettuce for the Tauranga Harbour
- First analysis of trace element speciation (trace elements-labile) in Tauranga Harbour and oceanic water
- Insight of trace element speciation using a novel system of classification (sustained -> unsustainable)

- Combining a range of analytical technologies to better understand the effects of pollution sources
- Providing valuable information for environmental managers
- Successfully worked in partnership with both regional councils and local iwi
- Provided insight into the complexity of trace metal pollution in a dynamic estuarine ecosystem and the disparate scientific approach associated with environmental monitoring
- Creation of a worldwide new environmental parameter “Ecohardness”

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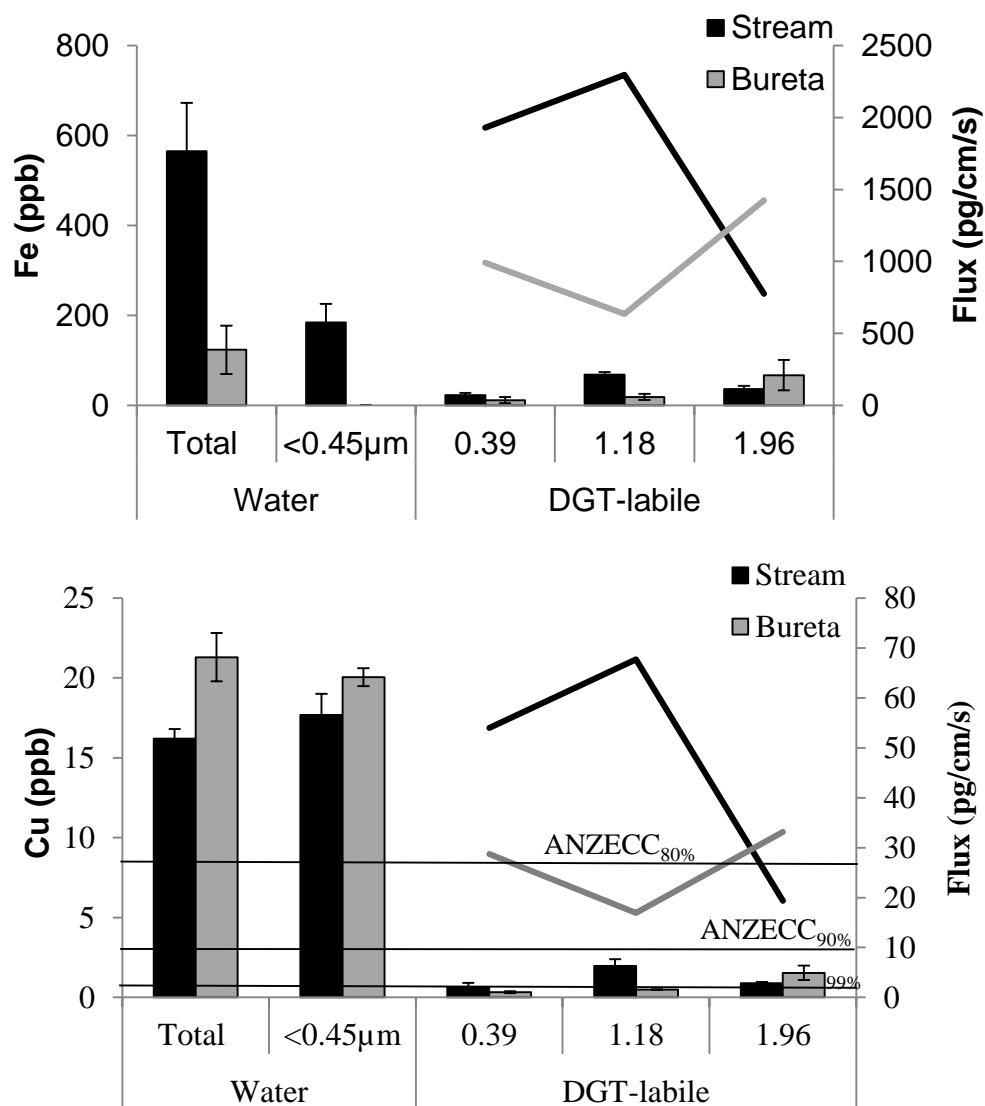
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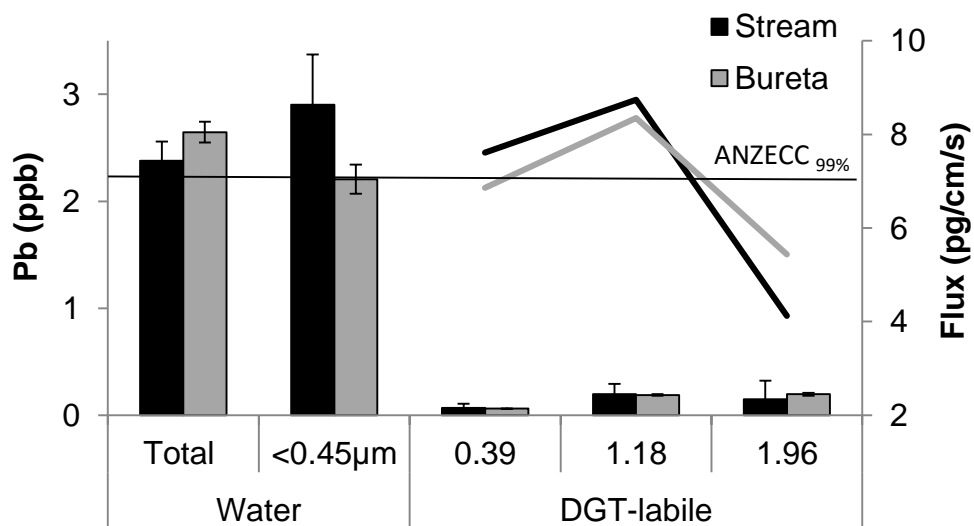
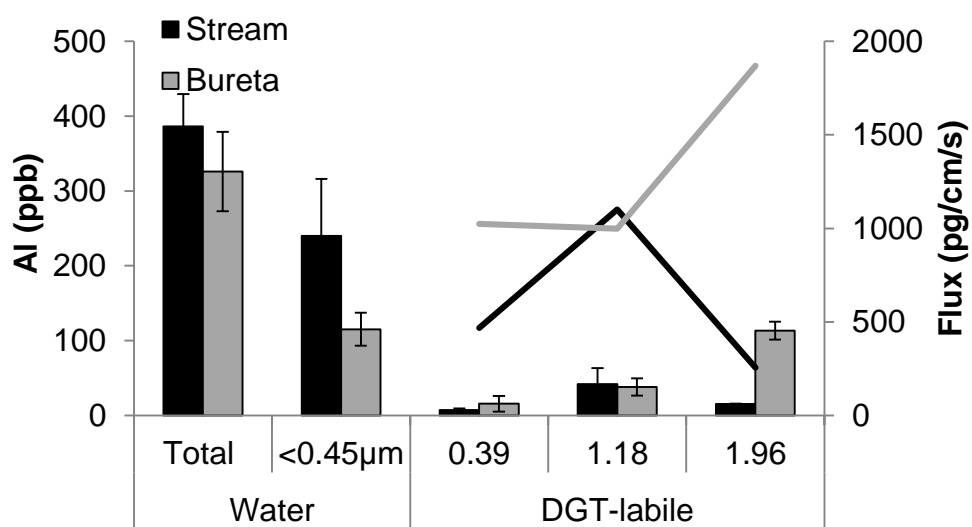
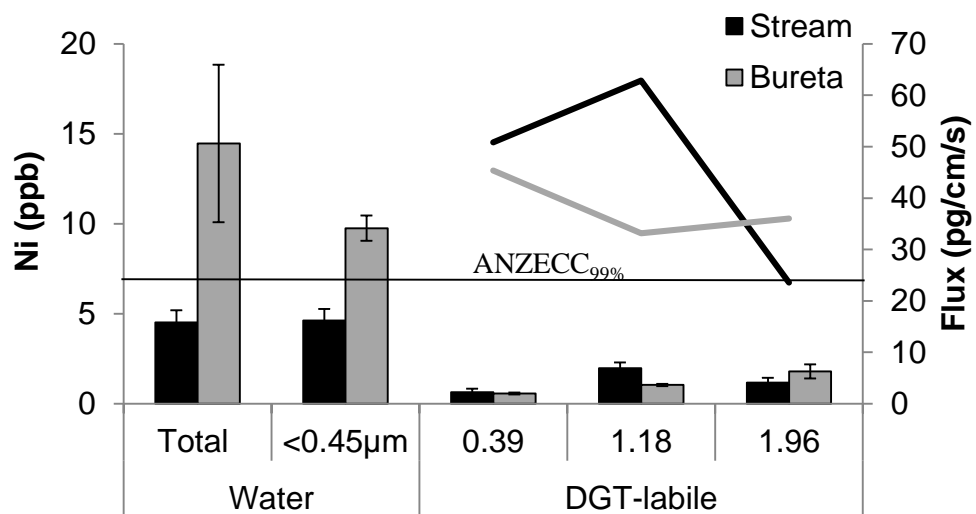
7 APPENDIX I

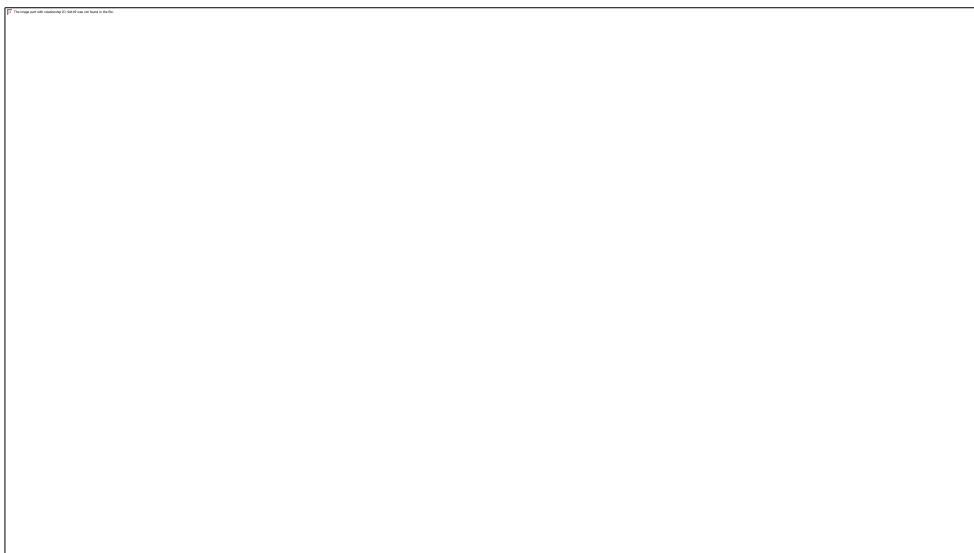
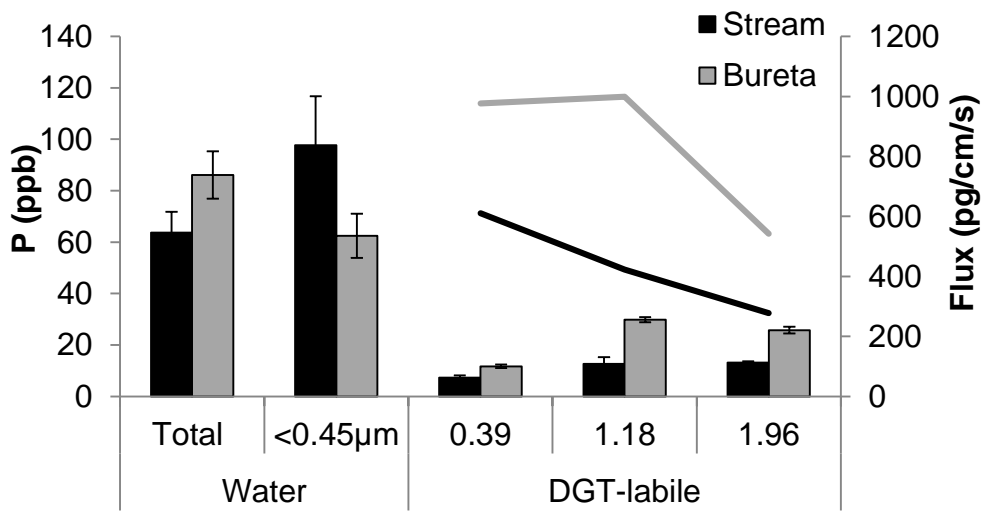
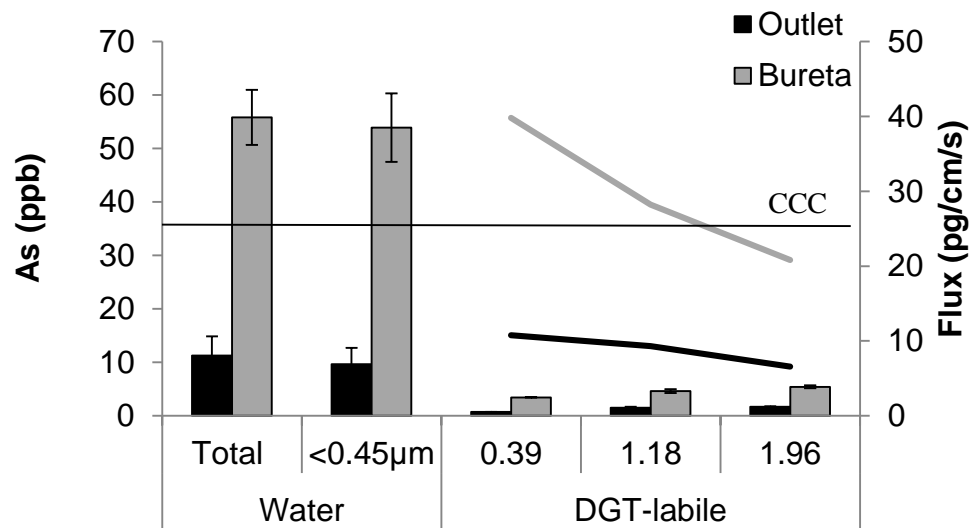
The data presented in Appendix I informed the discussion section in Chapter 3.

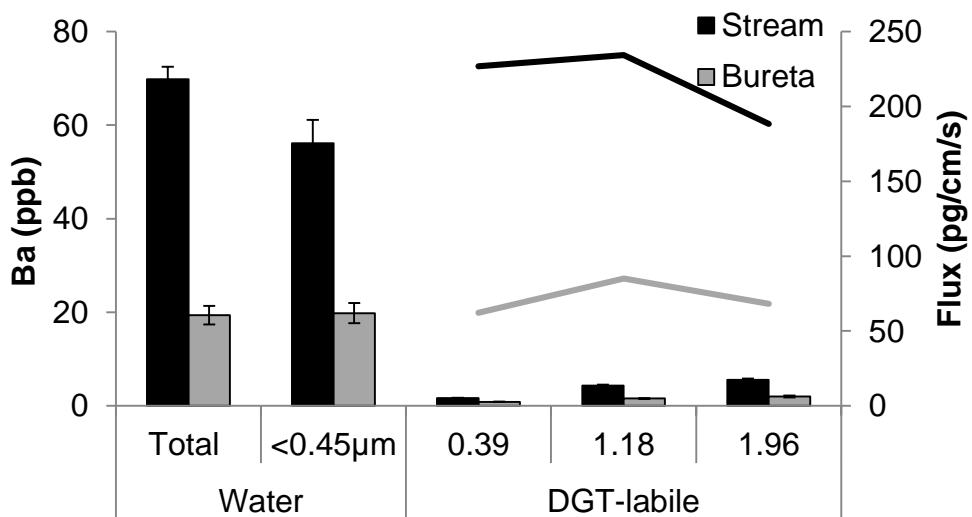
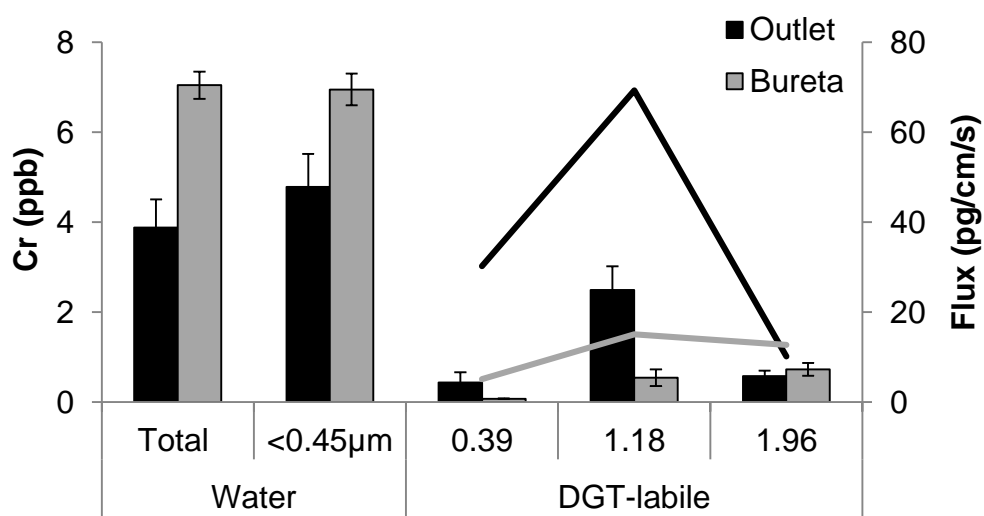
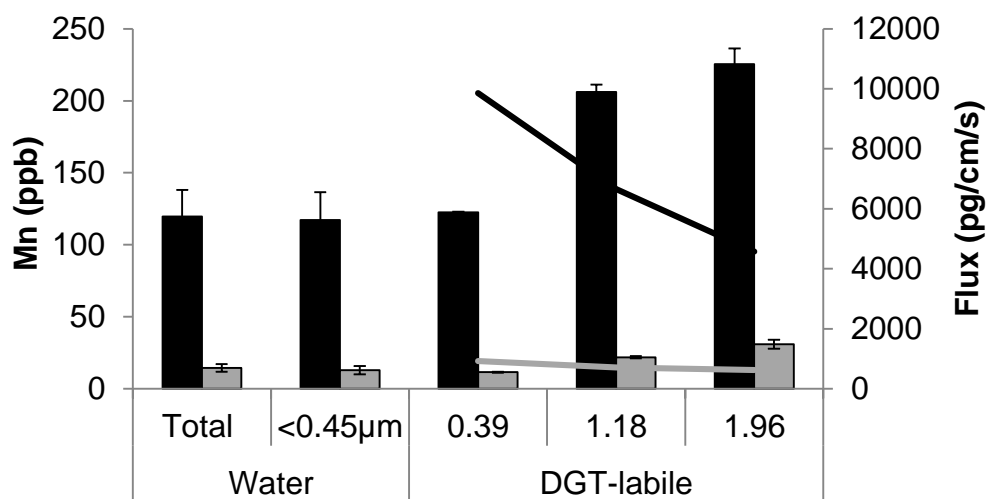
Results will be further explored for publication at a later date.

Fig. 7.1. Trace element concentrations in total, dissolved and DGT-labile fractions, and the associated DGT flux for different gel thicknesses (0.39, 1.18 and 1.96 mm). Zn, Cu, Ni and Pb were compared to safety guideline for ecosystem protection (ANZECC., 2000) and As to Criteria Continuous Concentration (CCA) (EPA 440/5-84-033., 1985)









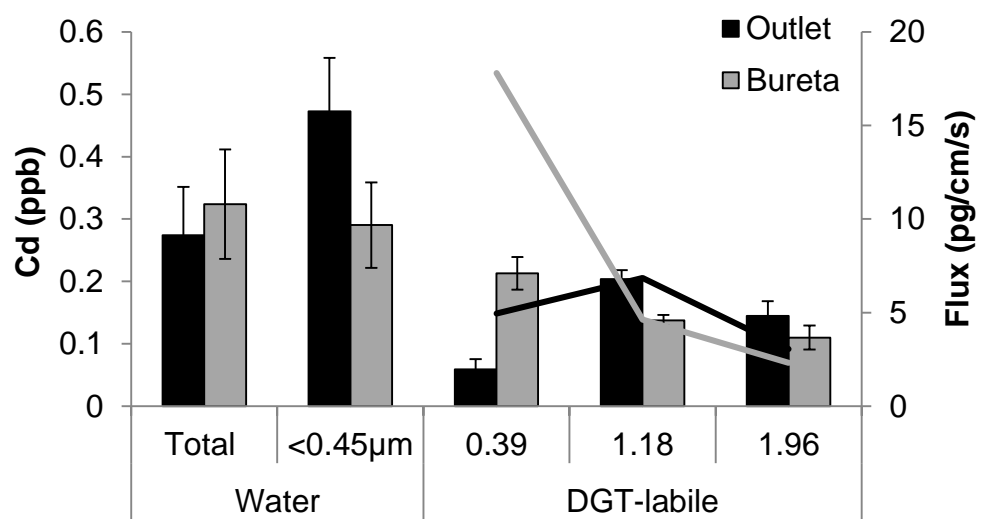
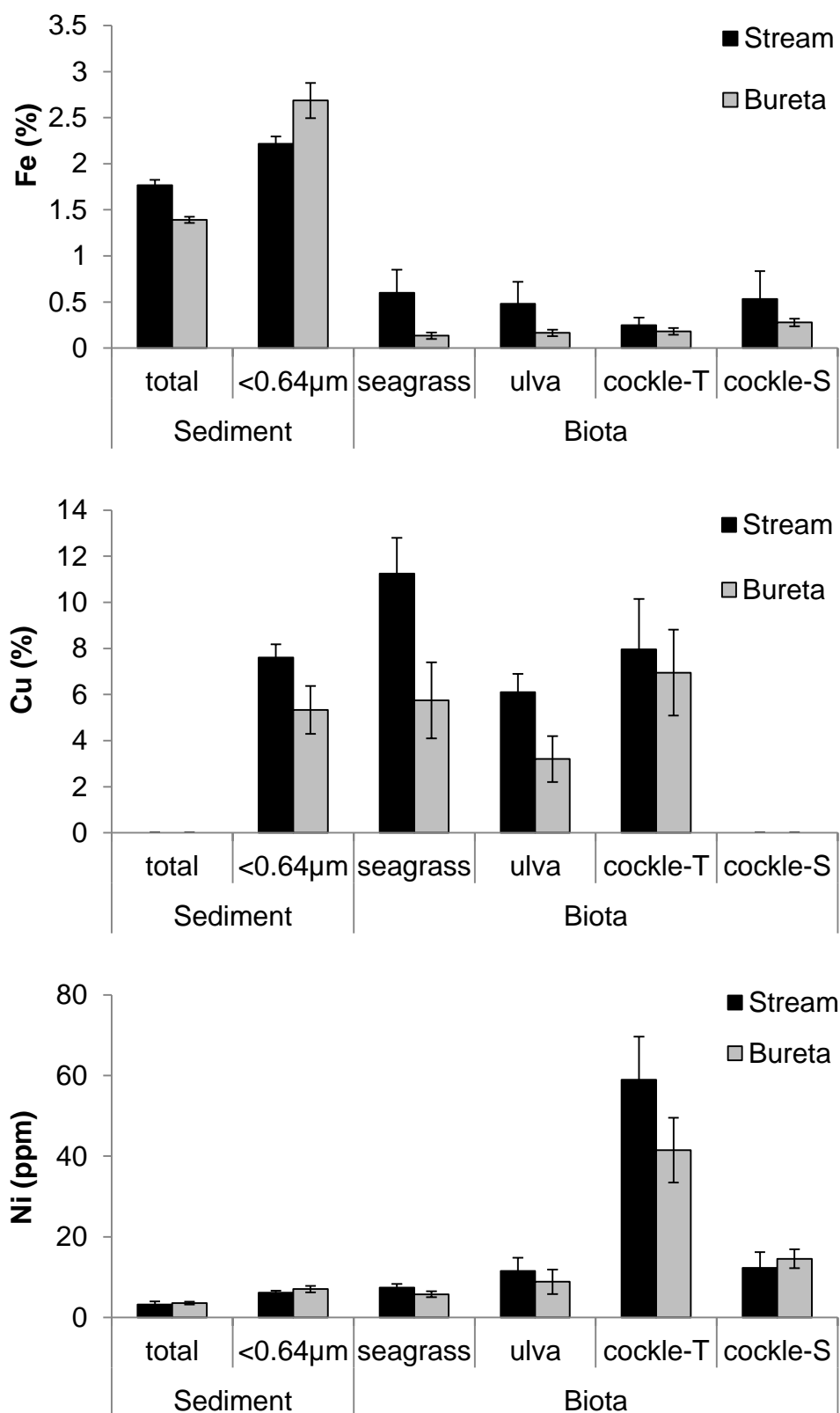
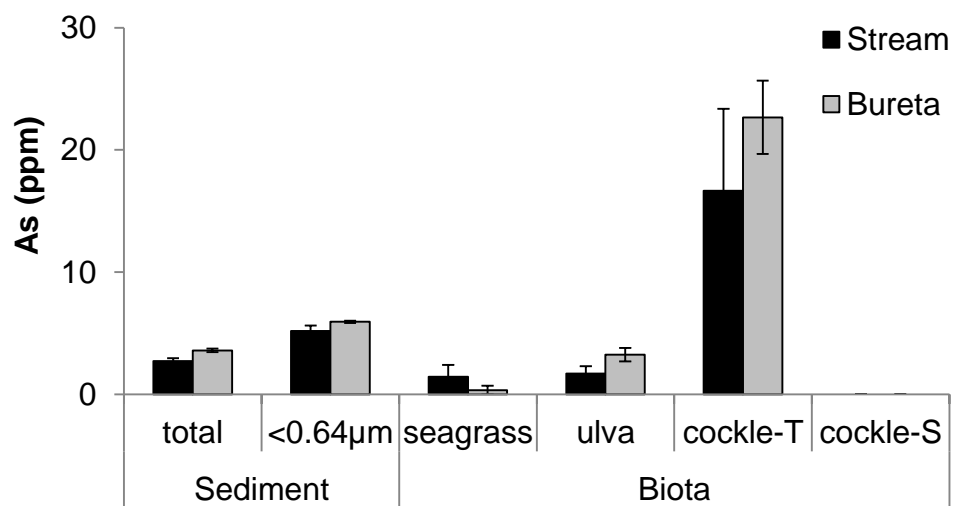
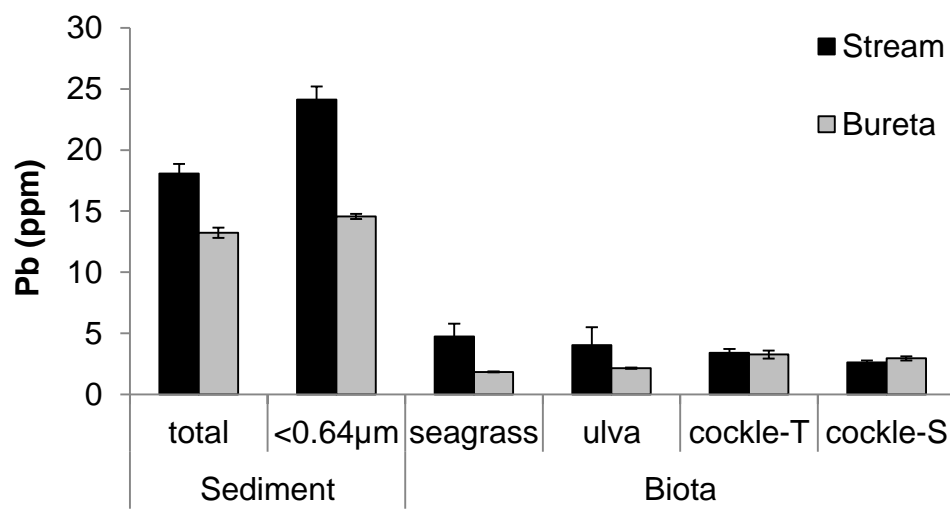
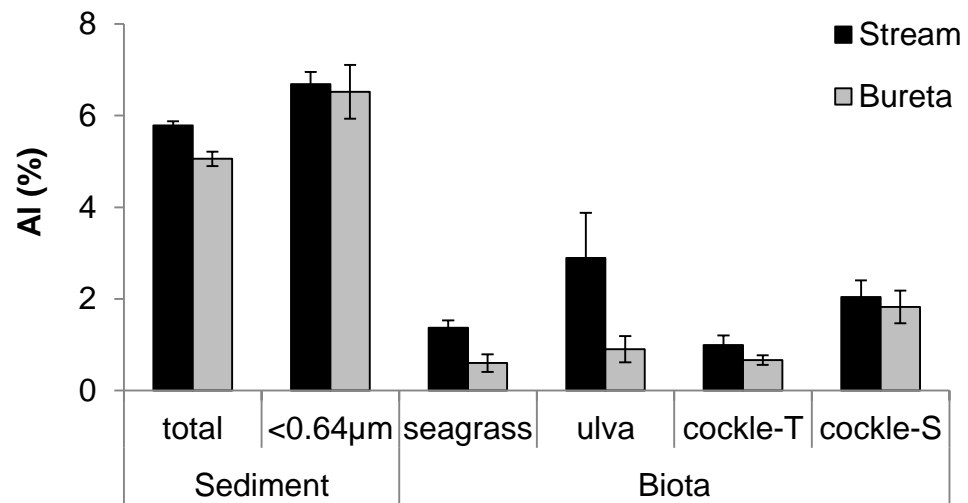
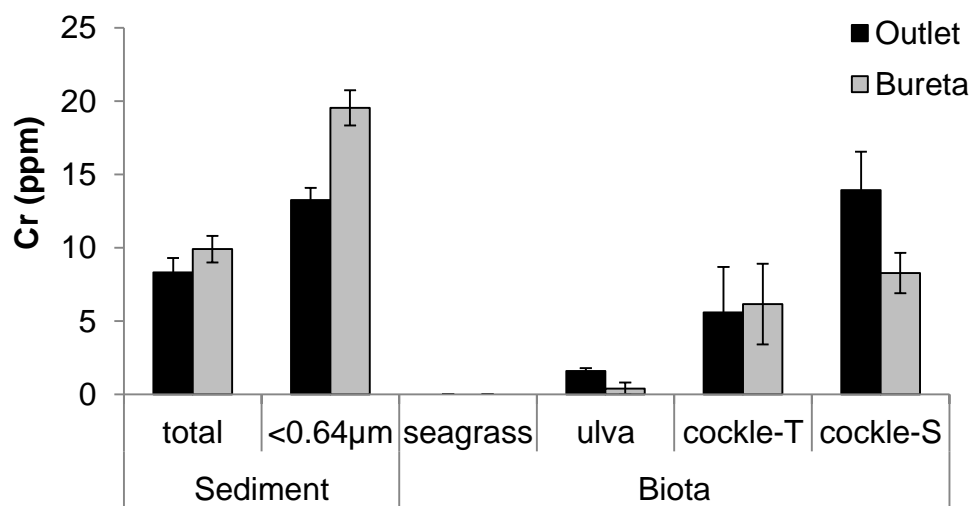
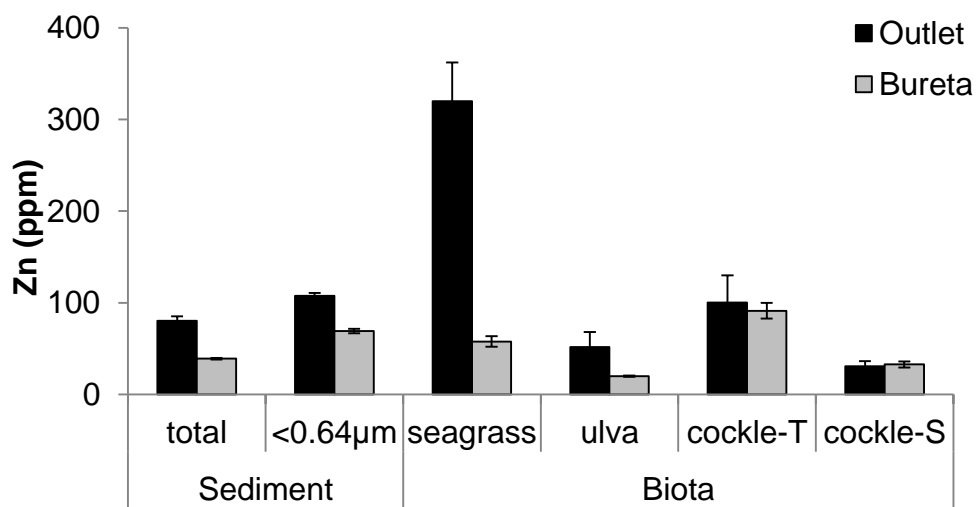
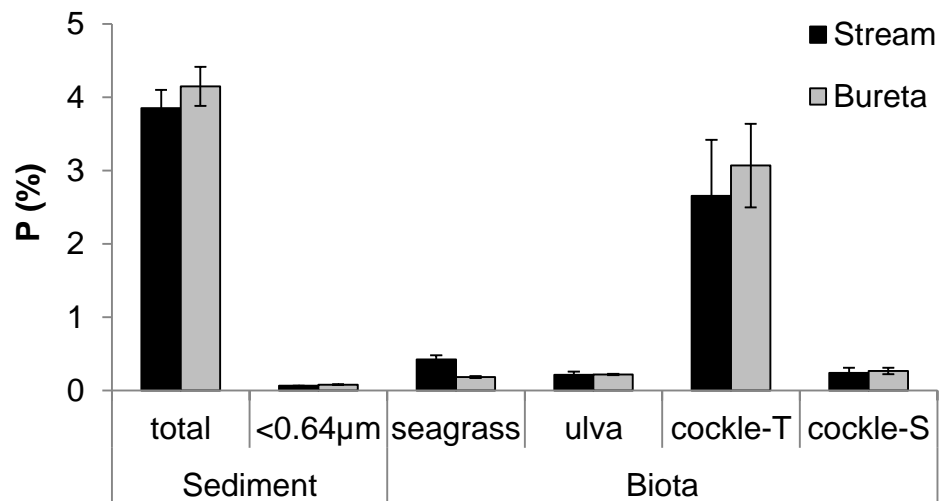


Fig. 7.2. Trace element concentration (ppm) in surface sediment (total and mud fraction), seagrass, sea lettuce and cockle tissues, and outer shell of cockles







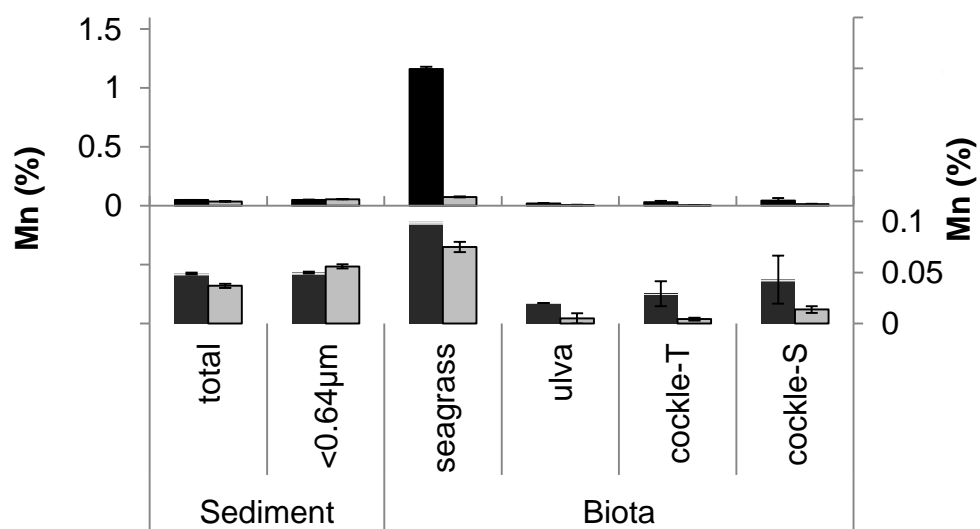
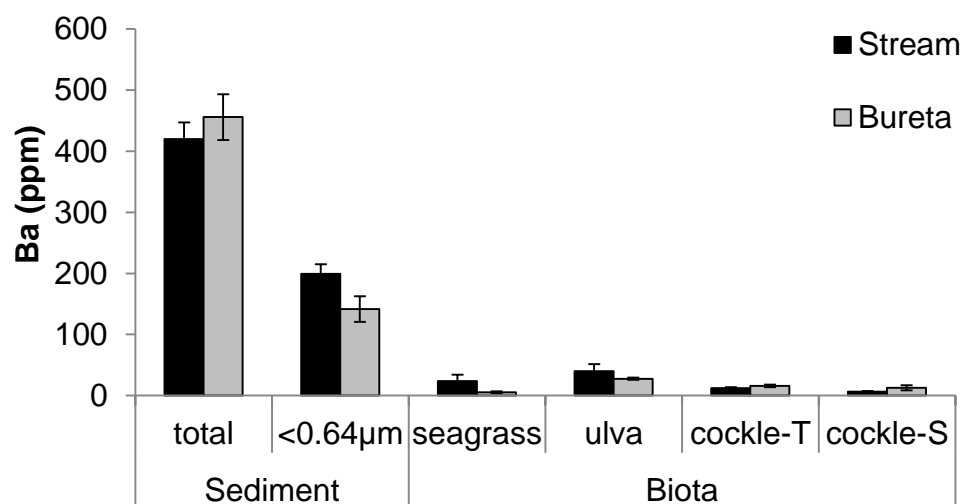


Fig. 7.3. Trace elements concentrations in seagrass, sea lettuce and cockle vs DGT labile

